A MANUAL
of the
ASPERGILLI

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PREFACE

Aspergillus as the name for a genus of molds dates back to Micheli (1729) but it was not until the middle of the 19th Century that the Aspergilli began to be recognized as active agents in many decay processes as occasional causes of human and animal disease and as fermenting agents capable of producing valuable biochemical products Various taxonomic efforts were made DeBary Fresenius van Tieghem and others described the particular species that they used Bainier described in brief inadequate terms all the Aspergilli he found Wilhelm and Wehmer reviewed the literature described and figured the forms they knew these were few in number but the work was done so well that it fixed group types In 1926 Thom and Church brought all of this material together in a monograph The increased study devoted to the Aspergilli in recent years shows some of their groupings to be inadequate In addition a large amount of new material has accumulated The Aspergilli have become increasingly important as responsible agents in a number of industrial fermentations Many of them are being found capable of producing antibiotic substances and their possible use in this field will undoubtedly be exhaustively explored For these reasons the need for a manual for those who wish to identify Aspergilli under observation without regard to the historical aspects of the group has become increasingly apparent

This book is definitely a manual not a monograph It is based upon comparative study of thousands of strains of Aspergilli in culture Representative strains giving the range of morphology and biochemical activity in each species are maintained in the permanent collection of the Northern Regional Research Laboratory Consistent efforts have been made to obtain the organisms actually used by authors who have put forward new nomenclature The manual thus seeks to present under species names only living cultures known to the authors although it seemed advisable to make a few additions based upon literature The species included are arranged as far as possible into natural groups which bring together aggregates of strains or species agreeing in important morphological characters For the most part physiological or biochemical information if available indicates related activities within these groups The names selected for use appear to be taxonomically correct A large number of names are necessarily rejected If known to belong to some unidentifiable member of a group or believed from literature to be correctly placed there each of these names is accounted for in the discussion of the group If the information available does not justify allocation to some species or species

aggregate, the name will be found in the check list with any information at hand Large gaps in our information about the Aspergilli still exist Some of these are pointed out in the text The great activity of the present day will undoubtedly render any arrangement of the Aspergilli obsolete sooner or later, but it is believed that the classification put forward here is at least temporarily practical

Recognizing that any species name for an Aspergillus appearing at any time in the literature may at some future time become important for some unanticipated reason an alphabetical check list giving each of the names found, the author, the date, and the place of publication has been included An index reference to page in the manual, or the method of disposal of the name, is added to bring the material to its greatest usefulness as a ready reference

Two types of bibliography are presented a general bibliography, alphabetical to author's name and sub indexed as to date of publication when necessary includes authors of species and other investigators whose work is cited in the text In addition a topical bibliography is presented Although incomplete it is hoped that the latter will assist greatly in the search for special literature on particular subjects Believing that the more recent literature on these subjects will generally be of the greatest interest and value the material is presented chronologically Duplication between the two bibliographies may or may not occur

A manual if it is to facilitate the identification of these molds by the actual worker in the laboratory, must present descriptive and illustrative material in as simple form as seems consistent with sound scholarship From our present point of view, the describer of a mold must know that mold in fruiting form under the microscope as known to the early mycologists and know it also in the culture tube It must be isolated as a pure culture and its life history and reactions followed out upon laboratory media Its definite place in some one of the aggregate species or groups of Aspergilli should be thoroughly established then by careful study and comparison proper nomenclature should not prove difficult

This manual then seeks to serve two purposes (1) to provide the worker encountering an Aspergillus with means for its identification and hence to open to him the whole literature of the group as well as the particular species, and (2) by enumerating all forms found in the literature and indicating their proper allocation to guide the user of that literature in the interpretation of names found in his reading but not known to him in nature in culture or in exsiccati

The authors acknowledge the cooperation of Dr Johanna Westerdijk Dr F H van Beyma and their colleagues at the Centraalbureau voor Schimmelcultures, at Baarn Holland in the free exchange of cultures and

information Professor Ph Biourge and Dr Paul Simonart at Louvain put their entire collection at our service after preparing and demonstrating their interpretations in their own laboratory Dr Raoul Mosseray sent his extensive series of variants in the *Aspergillus niger* group as accumulated from the Belgian Congo Dr Adalbert Blochwitz made many comments and criticisms in his numerous letters Professor Harold Raistrick and Mr George Smith submitted all strains reaching their laboratory with full notes on their own interpretations Cultures and photographs some of which are used in this manual have been furnished by Messrs John and Edward Yuill Series of cultures have been received from Drs Marie B Morrow and J J Taubenhaus in Texas Drs Roberta Ma and Y K Shih in China Drs G Kita R Nakazawa J Hanzawa and K Oshima in Japan Drs G R Bisby and G A Ledingham in Canada and Dr H Macy in Minneapolis as well as individual strains from many correspondents The laboratory collection owed much of its completeness to the punctilious workmanship and painstaking scholarship of Dr Margaret B Church

In the preparation of the manuscript outstanding contributions have been made by Dorothy F Alexander who prepared the line drawings and assisted generously in the checking and proofreading of the textual material by Mr Roland W Haines Photographer of the Northern Regional Research Laboratory who made all the color pictures as well as many of the black and white photographs and by Miss Nancy Brant who typed the manuscript in its final form

The authors are indebted to the Chas Pfizer and Company Inc Brooklyn New York for underwriting the cost of reproducing the natural color photographs

Administratively the preparation of this manual was made possible by the vision of Dr O E May Chief of the Bureau of Agricultural and Industrial Chemistry Mr H T Herrick Director of the Northern Regional Research Laboratory and Dr Robert D Coghill Chief of the Fermentation Division of the Northern Regional Research Laboratory who have developed the industrial exploration of the biochemical utilization of the fungi over many years

THE AUTHORS

PART I

GENERAL DISCUSSION

Chapter I

HISTORICAL INTRODUCTION

Historically the Aspergilli as a part of moldiness of things have always been a factor in man s environment, but for ages were brushed away as white yellow green red or black mold with or without any attempt at interpretation After the development of the microscope men began to see structure Micheli (1729) distinguished conidiophores and heads He noted that the heads were rough the spore chains or columns producing an uneven surface hence he gave the name Aspergillus (rough head) He then marked with Latin phrases his sketches of differently colored moldy substances for example *Aspergillus capitatus ochroleucus* probably some strain of *Aspergillus ochraceus* *Aspergillus capitulo pulla* for a black form etc Other authors followed using much the same terminology but without illustrations definite enough to give knowledge of the structure of the heads Thus Haller in 1742 put what appears to have been *Sporodinia* into the genus as *A ramosissimus* etc There is just about enough certainty in the use of *A albus* *A niveus* *A capitulo pulla* *A purpureus* etc , to justify the continued use of the name Aspergillus after taking out of the aggregate the extraneous material thrown into it by the very scanty microscopic examination given by the early mycologists

Persoon (in the 1790 s) threw the Aspergilli into his polyglot concept *Monilia* based upon the production of spores in chains resembling strings of beads He made no record concerning their origin Then Link in 1809 went back to Micheli and based his rejection of *Monilia* upon the specification that the e chains of spores must have their origin in a head (capitulum)

Link failed to examine that head closely enough to keep out questionable forms although we know that in describing *A glaucus* he had under his microscope one of that group as it was found then and now upon partly dried herbarium specimens Correct interpretation of the structure of this head appears first in the work of Corda who began about 1828 to publish his studies of fresh material as seen under his microscope Up to about 1850 each worker was prone to look at his predecessor s descriptions and figures and either assign whatever he had to another man s species or conclude that each specimen he had was new and add another group of names Montagne complained (1856) that none of the descriptions written before Corda were identifiable while some of us are equally uncertain of our ability to interpretMontagne

DeBary's laboratory in the early 1850 s seems to have introduced sufficient culture of the molds found to form the beginning of a permanent literature This started with the recognition that the yellow perithecia called *Eurotium herbariorum* by Link, which developed among the heads of *Aspergillus glaucus* upon his herbarium specimens, were actually borne upon the same mycelium (fig 7) Fresenius Cramer, Wilhelm and Brefeld in Germany followed Raulin and van Tieghem in France developed the fermentation of the tannins in gall nuts to gallic acid in the 1860 s with comparative study of other molds as a corollary In 1880 in Paris Bainier began publishing his studies of molds as they appeared in pharmaceutical products He was followed by Gueguen the Sartorys, and others in France and somewhat later by Biourge in Louvain, Belgium

Wehmer, in Hanover began publishing his biochemical studies in 1891, which led him to develop his more pretentious monograph published in 1901 Blochwitz undertook to develop his system ' early in the new century, but the World War delayed its publication until 1929 Meanwhile Thom and Church beginning about 1910 had published *The Aspergilli* as a taxonomic monograph in 1926 Aspergilli were listed in cryptogamic floras lists manuals and special papers of many kinds over the whole period but critical discussions were few

In somewhat over 200 years, an enormous mass of Aspergillus literature has accumulated Justice to the writers at each stage in the development of our information calls for an analysis of the conditions which surrounded its development Practically all of the early literature was microscopical the worker confined his study to specimens brought in from natural sources, each of which was often assumed to be typical of some species Each worker used the microscope that he had at hand and the technique of study already known to him Life histories and comparative examination of material from many sources were disregarded Publications appeared as parts of floristic studies of particular regions as reports of organisms found in particular lesions of man or animals or as observed in special industrial connections After DeBary s group began to study organisms in comparative culture the number of publications began to increase rapidly By 1929–1930 Tamiya and Morita were able to cite 2 424 title of papers which in some way concerned the Aspergilli in their published *Bibliographie von Aspergillus 1729 bis 1928* A mathematical analysis of this literature was published by Tamiya in 1931 Referring to his table 1 71 titles appeared in the 125 years before DeBary s 1854 paper 73 appeared in the next 18 years preceding Brefeld s 1872 papers 236 in the next 19 years just preceding Wehmer s oxalic acid reports in 1891 All of this may be called the period of physiological morphology The remaining two thousand published between 1891 and 1928 represent the

pure culture period This may equally well be called the biochemical period

The taxonomic part of this literature was scattered through several languages and represented many schools of nomenclatorial thought The man who had seen only three or four Aspergilli found no difficulty in separating them Each used his own descriptive terms—adequate for his purpose but useless to the next man with different species Saccardo just published them all Critical analyses were not available

In *The Aspergilli* (1926) as a monograph Thom and Church sought to bring together all of this taxonomic literature as published before that date and to present a critical opinion as to the proper relationship of the species described whether retained in the genus or placed elsewhere Some 350 names were thus accounted for but the actual number of species accepted as known in culture or probably determinable from existing literature was given as 69 (p 252) These were more or less arbitrarily considered in 11 groups In undertaking to account for all the described forms it was deemed advisable to include in the various groups discussed many forms whose published descriptions were inadequate for positive identification but complete enough to indicate their affinities with known sections of the genus Citation of these species in the older literature might therefore be traced to group relationship and in that way correlated with more recent studies of the same or related organisms In addition certain names were listed as entirely unidentifiable and certain other forms as belonging to other genera

Various other proposals for this purpose have been made Blochwitz in 1929 published his long-delayed System und Phylogenie with interpretations and proposals for grouping quite different from those of Thom and Church Neill (1939) reduced the species recognized to the larger aggregates paying little attention to details of head and spore formation George Smith (1938) seeking industrial utility simplified his descriptions and introduced many photomicrographs He discarded the literature for the most part and undertook to guide the worker to the larger groups which could be located principally by color and shape of head as shown by his figures Dodge (1935) keyed all species whose names appear in medical literature from their descriptions but without studying them in culture

In 1939 Biourge prepared a manuscript analysis of the genus for the Third International Microbiological Congress in New York His associate Dr Simonart came to represent him but left because of the war The paper was not presented but was transmitted to us because return to the author was impossible Biourge died somewhat later His scheme of classification prepared in his last years is not presented because it contains many things too bizarre to do justice to a man who for many years was a master workman as well as a valued friend

Chapter II

CLASSIFICATION, GENERIC DIAGNOSIS, AND SYNONYMY

Class Ascomycetes
 Order Plectascineae
  Family Aspergillaceae
   Genus Aspergillus

Class Fungi Imperfecti
 Subclass Hyphomycetes
  Order Mucedineae
   Family Mucedinaceae
    Subfamily Aspergilleae
     Genus Aspergillus

The above classification follows Engler and Prantl Changes in the names of class order and family appear in various proposals without essential differences in placement G W Martin would replace the names Plectascineae with Eurotiales, Aspergillaceae with Eurotiaceae and Aspergillus with Eurotium in the plea that the first name applied to the ascosporic form determines the generic usage Since the group has too many common characters to be split to advantage and since the non ascosporic forms vastly outnumber the ascosporic it is better to forget Eurotium along with the technicality In this arrangement the name Aspergillus appears in its proper place among the ascosporic fungi It also appears among the Hyphomycetes properly keyed to facilitate the identification of organisms obviously related but which do not produce ascospores as far as known

GENERIC DIAGNOSIS

There is progressive need for broadening the application of the name Aspergillus to include organisms whose structures as determined in culture by microscopic study point to membership in specific natural groups There is need for analysis of the question whether the whole group shall be retained as *Aspergillus* or further divided into more closely related entities such as *Eurotium* of Link *Aspergillopsis* of Spegazzini *Diplostephanus* of Langeron *Sterigmatocystis* of Cramer and perhaps others There are so many arguments for keeping them in a single group that the characterization of the genus *Aspergillus* used by Thom and Church in

1926 has been emended and introduced here This is followed by brief considerations of the other more significant synonyms

Aspergillus Micheli in Nova Plantarum Genera p 212 Plate 91 1729
Compare Link, in Obs p 16 1809 Corda in Icones Fungorum 4 31 Tab VII fig 94 1840 and Thom and Church in The Aspergilli p 4 1926

Vegetative mycelium consisting of septate branching hyphae colorless bright colored or in a few forms slowly becoming brown in localized submerged areas or producing brown crusts or sclerotia conidial apparatus developed as conidiophores and heads from specialized enlarged thick walled hyphal cells (the foot-cells) producing conidiophores (stalks) as branches approximately perpendicular to the long axis of the foot-cell and usually to the surface of the substrata in or upon which they are borne conidiophores unseptate or septate usually enlarging upward and broadening into elliptical hemispherical or globose fertile vesicles bearing fertile cells or sterigmata either parallel and clustered in terminal groups or radiating from the entire surface sterigmata either in one series only or as a primary series each bearing a cluster of two to several secondary sterigmata at the apex conidia varying greatly in color size shape and markings successively cut off from the tips of the sterigmata by crosswalls (not produced by budding) and forming unbranched chains arranged into radiate (globose) heads or packed into columnar masses perithecia found in certain groups only unknown in most species cleistocarpic thin walled producing asci and ascospores within a few weeks sclerotia regularly found in some strains occasionally found in other strains and not found in other and closely related strains mostly globose or subglobose composed of polyhedral thick walled cells

Eurotium Link in Obs p 31 Taf 2 fig 44 1809

Synonym *Mucor herbariorum* Wiggers in Primitiae Florae Holasticae as No 1158 1780 See also DeBary in Bot Ztg 12 425 1854

The yellow perithecia suspended in networks of hyphae above or at the surface of his badly dried herbarium specimens were taken by Wiggers (1780) as the basis of *Mucor herbariorum* Link (1809) recognized the bodies as ascosporic hence segregated them under the generic name Eurotium Then in 1854 DeBary publi hed proof that these perithecia were borne upon the same mycelium as the a exual *A glaucus* fruits among which they developed He then called each of his Aspergilli Eurotium Aspergillus followed by the specific name whether ascosporic strains were known or not In pite of technicalities invoked by some to bolster the

use of the name Eurotium for all Aspergilli, or failing in that for all ascosporic strains most workers have accepted the numerical predominance of the non ascosporic strains as ample reason for the general use of the name Aspergillus For practical purposes Eurotium is not used here

Sterigmatocystis Cramer, in Vrtljschr Naturf Gesell Zurich Jahrg 4, Heft 4 p 325 Taf II, figs 1–15 1859

Cramer in 1859, published his study of a black Aspergillus from the human ear Since the fruiting head differed from that of Fresenius' *A fumigatus* by showing a primary series of sterigmatic cells radiating from the vesicle, each bearing a crown of several sterigmata which in turn each bore a chain of spores he made this character the basis of his new genus *Sterigmatocystis* Cramer s name has been accepted by many workers, but was rejected by Wehmer Thom, and others on the proof that such use would separate strains obviously related in such a group as *Aspergillus flavus* and its allies and even among the black Aspergilli studied by Cramer himself The additional name serves no useful purpose as an aid to identification, hence is not recognized here

Euaspergillus Ludwig, in Lehrbuch des niederen Kryptogamen p 258 1892

The proposal to apply a separate generic designation to all Aspergilli producing sclerotia would take out the groups typified by *A candidus*, *A niger*, *A wentii*, *A tamarii* *A flavus* and *A ochraceus* No one has followed Ludwig

Aspergillopsis Spegazzini in An Mus Nat Buenos Aires Ser 3 13 434 1911

The black spored Aspergilli were described as dematiaceous hence separated from all the other groups No practical reason for accepting this proposal has been offered

Diplostephanus Langeron in Compt Rend Soc Biol Paris 87 343-345 1922

Under this proposal ascosporic Aspergilli with the double series of sterigmata would be separated with *A nidulans* Eidam as type No technical application of nomenclatorial rules justifies the complications introduced

In addition to the above names proposed to cover blocks of species with particular characters in common, a series of names have been used

by various authors for individual species *Alliospora* for a black form *Ascophora nigrans* for *A niger* *Aspergillopsis* Sopp for an unidentified organism *Cladosarum* for Yuill s mutant of *A niger* *Dimargaris* for some white forms *Emericella* and *In engaea* for *A variecolor* *Mucor* as a place to assign *A herbariorum* *Sartorya* for a possible ascosporic *A fumigatus* These names are cited in the check list but contribute nothing to this study of the group as a whole

Chapter III

MORPHOLOGY AND DESCRIPTION

INTERPRETATION OF PUBLISHED DESCRIPTIONS

Basic Assumptions In interpreting the descriptions of Aspergilli in a literature covering a long period of time, certain assumptions although *not always justified form a working hypothesis for presumptive identification* Conidiophore walls and conidial walls are assumed to be colorless and smooth unless color or markings are either figured or described Perithecia and sclerotia are assumed to be lacking unless the presence of *such structures is specifically noted Colors are assumed to apply to the* general color scheme of the colony unless specifically applied to the conidia ascospores or other details by the describer Whereas colony coloration may arise from an admixture of conidial structures and varying amounts of *vegetative hyphae colored or uncolored together with perithecia* or sclerotia in greater or lesser numbers it is assumed to result from the massing of conidial heads unless otherwise stated

Difficulties encountered in interpreting descriptions based upon color are less for the worker with a growing culture before him than for the one handling descriptive literature alone, since the presence of white green yellow green, brown or black heads is readily distinguished with a handlens even though sparingly produced upon a colony in which another color predominates as in many members of the *A glaucus* group or in *A flavipes* The color of the conidial heads is often made the primary basis of species description

Extent of Study Interpretation of descriptive literature accompanied and supplemented rather than preceded the study of great numbers of cultures so that the groups established are based upon the *actual handling* of thousands of cultures representing hundreds of forms of Aspergillus handled during a period of more than thirty years Many of these were studied on natural substrata before their isolation In addition exami*nation of exsiccati from several large herbaria while more or less* unsatisfactory as to detail in identification of species furnish confirmatory evidence of the soundness of the groupings proposed

Types For a few of the specific names in use today the type strain has been definitely maintained in culture For most series selection of a morphological entity to give a concrete concept back of the use of a name becomes a matter of critical judgment For the purposes of this manual an attempt has been made to base the use of the individual name upon the

morphological picture most frequently encountered rather than upon a selected strain assumed to be but not known to be the one first described

Descriptive terms For purposes of description a standardized use of terms has been adopted Great diversity is encountered in the literature in various languages Even translated into Latin Saccardo never homogenized the terms so that succeeding descriptions upon the same page use descriptive terms in the same sense To make comparison with existing literature more convenient the usages defined in the following pages will cover the morphology as definitely as possible and indicate the usages found in the older literature To serve as a basis for the collection interpretation and presentation of pertinent information regarding Aspergilli to be studied a guide sheet of the type used by the authors is presented in the introductory portion of the manual proper (p 82)

THE ASPERGILLUS COLONY

Since few of the Aspergilli regularly produce perithecia and ascospores a basis for identifying the majority of the molds of this group as they are actually encountered in nature and in culture must be found in the description of the colonies and in the details of morphology found in the spore-bearing structures available

The vegetative mass of most Aspergilli consists of submerged mycelium from which only fruiting hyphae rise above the surface Such colonies suggest a field of ripening grain in which conidiophores and ripening heads predominate and have been described as velvety (the German term used is *rase*) from their appearance in many species Some species produce a more or less aerial felt (floccosity) of branching and interlacing hyphae bearing conidophores This is characteristic of certain strains of the *A versicolor* and *A fumigatus* groups upon Czapek s solution agar and other culture media commonly employed and it is normally one of the most striking characters of *A wentii* under laboratory cultivation (fig 1 B) Many strains of the *A glaucus* group form long streamers of hyphae hanging from meat stored in cool damp rooms and certain of these retain this character in laboratory culture The character of the surface growth is a diagnostic characteristic which is usually fairly reliable under reasonably uniform conditions of culture

In describing the Aspergillus colony cognizance should be taken of such factors as age rate of growth temperature of incubation and the composition of the substratum Provided with this information subsequent investigators can intelligently interpret their cultures in terms of species previously described

Many species and strains of Aspergillus often produce conspicuously zonate colonies Most commonly these take the form of fairly regular

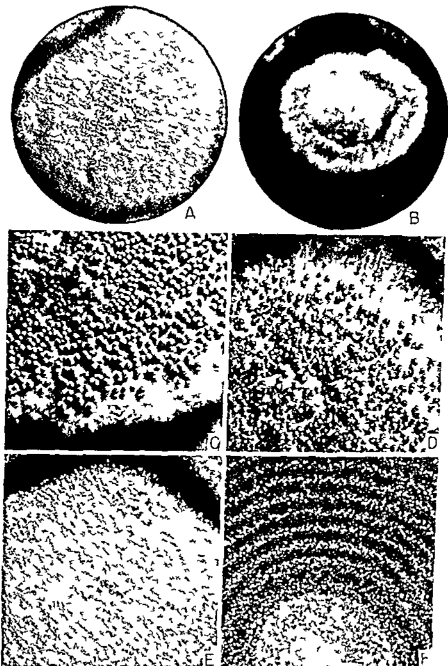


Fig 1 Colony types in the Aspergilli *A Aspergillus parasiticus* NRRL No 465 heavy sporing non floccose colony on Czapek s solution agar room temperature 10 days × 2 *B Aspergillus wentii* NRRL No 375 light sporing floccose colony grown under the same conditions × 2 *C Aspergillus variecolor* NRRL No 1954 characterized by the production of abundant large perithecia × 3 *D Aspergillus alliaceus NRRL No 315 characterized by abundant black sclerotia* × 2 *E Aspergillus ochraceus* NRRL No 408 on Czapek s solution agar showing heavy sporing essentially azonate colony × 2 *F* The same on hay infusion agar strongly zonate × 2

as in *A niger*, or the heads may be uncolored or nearly so, while the outer layers of the conidiophore wall may be colored as in *A flavipes* and *A ochraceus* In some species of the *A glaucus* group, the colony color is at first green with the development of conidia, then predominantly yellow to ferrugineous from ripening perithecia Again, in other species of the same group the walls of the conidiophores and, more particularly, aerial hyphae become encrusted with granules that are characteristically yellow in the young colony, but become reddish or ferrugineous in age Such colonies are at first predominantly yellow with green heads inconspicuous on a yellow background and later become rusty red or brown

In the *A flavus* group, Saito (1907) followed by Thom and Church (1921, p 115) found that cultures with brighter shades of green when subjected to a vapor of ammonia would lose the green color and assume the somber yellow shades of variant members of the same group and that this reaction was reversible, since the vapor of acetic acid would restore or even intensify an original green shade The experiments pointed to the hypothesis that the wide range of shades produced by mixtures of yellow and green in the *A flavus oryzae* group may be attributed to racial limitations strain by strain, in the range of hydrogen ion concentration produced by metabolism When a carbohydrate fermentable by the particular species is present an acid reaction is promptly produced in the growing colony, as growth progresses alkaline products are also produced The colony color in this series, therefore reflects first the intensity of the initial acidity as shown by the intensity of the green color reached The persistence of this shade or its subsequent reduction or entire disappearance to leave a somber yellow or finally brown colony represents the balancing of the two activities As a result, certain strains of this group if grown on Czapek s solution agar, are *quickly and very persistently deep green others become particular* shades of green which fade to yellow and finally some of them to brown and a few forms produce no true green color but assume a somber yellow with the first development of conidia

Color changes in the conidia have not been satisfactorily worked out In the *A niger* group the shade of yellow to purple-brown or black seems to be a strain or race character little influenced by handling which is not destructive of the racial entity Each race seems to reach a fixed quantitative limit in the secretion of the coloring substance thus reducing the most conspicuous diagnostic character among closely related forms to a quantitative rather than a qualitative basis of separation

Color in Conidial Walls

The conidial walls may be smooth but carry sufficient coloring matter in diffused form to give the characteristic colony color Within such

series as *A fumigatus*, *A nidulans* and *A ochraceus* however strains or races may be found which vary from conidial walls smooth or nearly so to walls bearing echinulations or even traceries apparently produced by aggregation of color substance into spinules or bars between the outer and inner walls of the cells Although smoothness and echinulation have received much weight in descriptive literature observations such as the above would indicate that this character should only be used to separate nearly related strains in the same group rather than as a group character

Literature on the nature of color in Aspergilli seems to begin with Linossier's study of aspergilline as produced by *A niger* in 1891 this was followed more recently by Quilico and Quilico and Di Capua in 1933 Disregarding the record of observations only more pretentious work appeared when Bainier and Sartory undertook to use color production to separate members of the *A glaucus* group about 1910 to 1912 Their experiments supplemented observations of colonies checked against a color chart with a routine series of solubility and precipitation tests Blochwitz (1929-1935) followed by using a series of routine solubility tests against all species producing bright colors but failed to coordinate his tests to show the relation of test to culture medium and conditions and to age of the culture studied Later Gould and Raistrick (1934) and Raistrick Robinson and Todd (1937) studying the *A glaucus* group extracted and defined the colors found but again failed to follow the transformations in the color of the particular species during the course of colony development Until someone correlates color determination and composition more closely color observations will continue to be useful accessory data which must be related to the age of the colony and to the composition of the medium as closely as possible to have value

Colors in the Substratum

Production of bright colors in the substratum is frequent among the Aspergilli The color produced by any species or race in any medium is dependent first on the ability of the mold to elaborate the particular product and second on the presence of the necessary building material in the substratum A mold may grow well upon a particular medium without discoloring it a transfer from this colony to another substratum may turn the second medium red or yellow

Color in the substratum is the result of the particular Aspergillus acting upon the particular medium under a certain range of temperature Most of the species of Aspergillus if they produce any color produce from a trace to abundant yellow in the early stages This may persist or give place to shades of orange red or purple In some cases the color fades out as the colony becomes older In descriptive work progressive changes

in intensity of color, or the presence or absence of color in different sub strata, make the use of closely defined shades or intensities of color in the substratum an unreliable means of characterizing cultures

Observations of colors produced in thè substratum remain, however, very conspicuous and exceedingly useful accessory characters which aid in the placing of species The describer must bear in mind that such color reactions are confirmative not absolute characters in separating species

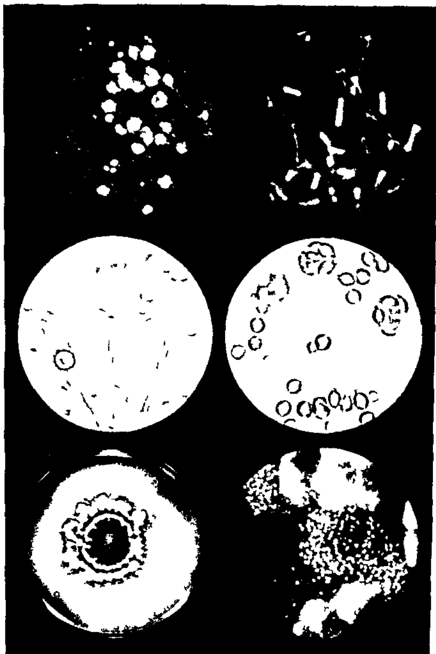
The final difficulty in dealing with color as a separating character rests in the loose use of color names, which is only partially corrected by the use of color standards Comparison of the same culture by different individuals introduces very considerable discrepancies which become serious when a specific descriptive name or number from one of these standards is introduced into a technical description In general, a series of observations giving a range of colors for a species is less liable to introduce errors of subsequent identification

MORPHOLOGY

From the time of Micheli, the name Aspergillus (literally, rough head) has been used for molds with a conidiophore or stalk and spore bearing head (capitulum)

The Head

The first structure observed in a detailed study of the colony is the spore-bearing head The color, shape size and arrangement of such heads are characteristic of the species and to a lesser extent of the groups to which they belong (figs 4 and 5) Wehmer (1901) roughly grouped his Aspergilli into Microaspergilli and Macroaspergilli on the basis of the size of the fruiting parts Thus *A fumigatus A nidulans A sydowi*, and *A versicolor* would represent Microaspergilli while *A niger*, *A clavatus*, *A ochraceus*, *A wentii* and *A tamarii* would be readily classed as Macroaspergilli The distinction breaks down when great numbers of forms are studied, but the comparative size of heads and conidiophores remains a useful adjunct in description The heads in certain species show a consistent range of measurements and form, as in *A fumigatus and A nidulans* which have heads of small diameter forming columnar masses (fig 4) Similarly characteristic heads of *A niger A ochraceus*, or *A wentii* are globose and large (fig 5) In other species notably in *A flavus* or *A candidus* (fig 60) several sizes and shapes of heads are regularly found in the same colony The range of size and shape, however, remains characteristic The observation of many heads in the colony and pref erably in many separate cultures forms a better basis for description of sizes and measurements than limited observation Further description



PL. I

A–D. Aspergillus ... *dul...* (T...d...) W... NRRL N... 191 ... *A* (upper left) portion of colony ... d... ...g ... heads ... *B* (upper right) conidial heads ... columnar ... × 60. *C* (center left) photomicrograph of conidial heads ... brown ... phores ... × 500. *D* ... conidiophores ... × 100. *E* ... *F*. *Aspergillus* ... NRRL N... 1... *E* (lower left) ... colony ... stalk ... central area ... stalk ... yellow ... floccose ... composed ... Hülle cells. *F* (lower right) portion of same colony ... enlarged × 8. (Color photographs by H... North... Regional Research Laboratory. Reproduced through co-operation of Chas. Pfizer & Co. I...)

of the structure of the head is given in the more logical order, following the discussion of the stalk or conidiophore

The Foot Cell

The first step toward conidium formation in the Aspergilli is the differentiation of certain cells (the foot-cells) in the mycelium for propagative purposes These cells become larger thick walled and each usually bears a single conidiophore as a branch perpendicular to the long axis of the cell (fig 2 A) and usually about midway between the ends of the cell In age these cells frequently become fantastically curved and twisted with their connection to vegetative hyphae inconspicuous but usually still determinable These foot-cells are commonly submerged in the substratum although there are a number of strains of the *A glaucus A fumigatus A versicolor* and especially of the *A flavus-oryzae* groups in which the conidiophores arise in this way from aerial hyphae In *A effusus* the foot-cells are frequently long and several of them connected together to form whole hyphae bearing considerable numbers of very short conidiophores (fig 71 B_1) hence their differentiation from the sterile or vegetative cells is less easily determined Failure to recognize the foot-cells as present in the Aspergilli led Ferdinandsen and Winge (1920) to describe *S dipus* using the foot-cell as the principal diagnostic character of the species The presence of such a differentiated foot-cell is proposed as an arbitrary character to be used in separating certain depauperate forms of Aspergilli which approach the structure and appearance of the monoverticillate Penicillia (Citromyces) from the Penicillia Organisms which lack the typical Aspergillus head with its conidiophore and especially its foot-cell may be best classified elsewhere

The Conidiophore or Stalk

The erect perpendicular branch from the foot-cell constituting the conidiophore usually enlarges upwards toward the apex at which it dilates more or less definitely to form the vesicle (fig 2 C–E) The section of the conidiophores from the foot-cell to the base of the conidial head is measured and reported in describing species

The conidiophore in some groups is not only septate but each cell is sufficiently distinct to justify the term *articulate* which is frequently encountered in the older descriptions In our experience in examining specimens articulate conidiophores are found only in the *A glaucus* group In most Aspergilli the unity of the whole conidiophore is fairly accentuated Septa if present are thin fragile and inconspicuous the whole conidiophore is enclosed by continuous characteristically thickened walls without conspicuous nodes as an evidence of septation

Thickening of the conidiophore wall may be uniform or may be greater at the base, thinning to negligible toward the apex Two general sections

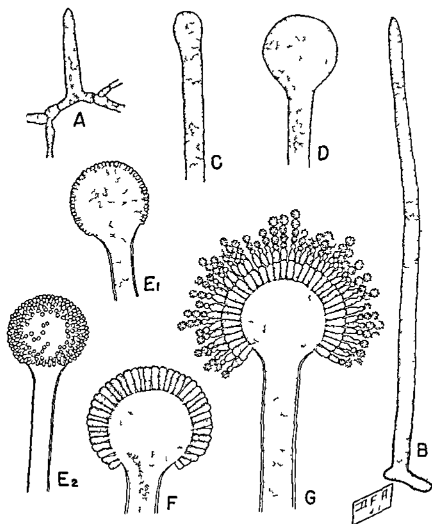


FIG 2 Development of the conidial apparatus in *Aspergillus niger* *A* Foot cell bearing young conidiophore as a vertical branch *B* Developing conidiophore × 172 *C* and *D* Development of the vesicle by swelling of the terminal portion of the conidiophore × 265 E_1 and *F* Vesicle in optical section and surface view showing early development of primary sterigmata × 265 *F* Later stage in development of primary sterigmata × 265 *G* Young fruiting head showing secondary sterigmata bearing chains of conidia × 265

based upon the character of the thickening of the conidiophore wall are fairly readily distinguished although the careful use of high magnification is occasionally necessary to separate certain strains In the first the

outer surface of the wall is free from pits warts or roughenings hence is called smooth its structure is difficult to differentiate the mass of the cell wall appears homogeneous or nearly so under the microscope and does not absorb the ordinary protoplasmic stains In some species the inside

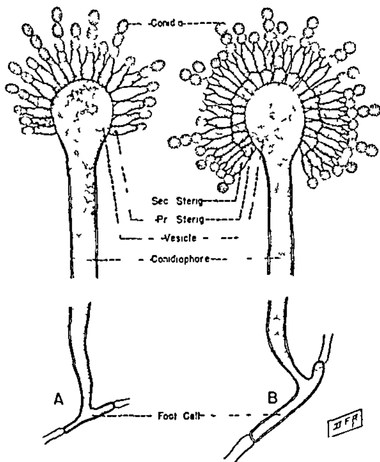


Fig 3 4 *Aspergillus niveo glaucus* NRRL No 127 typical head showing only one series of sterigmata × 575 *B A versicolor* NRRL No 239 typical head showing sterigmata in two series × 1?00

surface of the wall shows irregular clumps or uneven thickenings When broken many of these conidiophores show uneven or jagged ends like a broken glass tube in the *A niger* group the broken ends split like bundles of laths (fig 64 B) giving a possible clue to the method of their formation In the second section the wall appears dotted or pitted or as interpreted



FIG 4 Conidial heads group types *A Aspergillus clavatus* group conidial heads of *A giganteus* NRRL No 10 showing typical clavate form, × 15 *B Aspergillus glaucus* group heads of *A niveo glaucus* NRRL No 127 showing characteristic radiate pattern × 35 *C Aspergillus nidulans* group heads of *A nidulans* showing typical short columnar form × 35 (Photograph by Edward Yuill) *D Aspergillus flavipes* group heads of *A flavipes* NRRL No 1959 typically barrel form or loose columnar as shown × 18 *E Aspergillus terreus* group *A terreus* NRRL No 265 heads columnar of uniform diameter throughout often becoming quite long as shown × 22 *F*, *Aspergillus ustus* group *A ustus* NRRL No 1974 heads typically loose and radiate as shown under certain conditions approaching columnar × 22

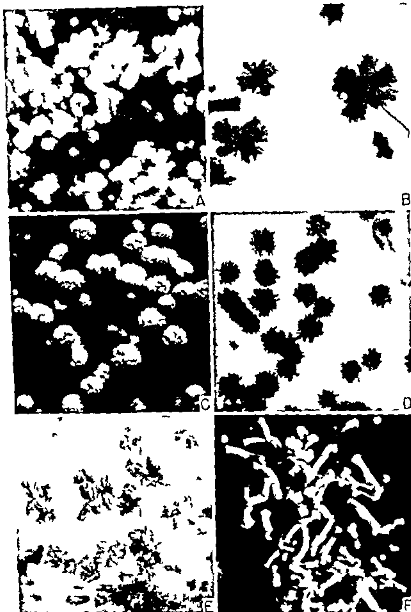


Fig 5 Conidial heads group types *A* *Aspergillus candidus* group *A candidus* NRRL No 308 showing characteristic mature heads of different dime sions × 18 *B* and *C* *Aspergillus niger* group *B* strain NRRL No 67 show typical mature heads × 30 *C* *A niger* mut *schiemanni* heads typically globose shown (Photograph by Edward Yuill) × 30 *D* *Aspergillus wentii* group typi globose heads of *A wentii* NRRL No 397 × 18 *E* *Aspergillus flavus-oryzae* grou *A flavus* NRRL No 1957 typical mature heads × 18 *F* *Aspergillus ochrac* group *A ochraceus* NRRL No 398 typical mature heads splitting into diverge columns of conidia × 18

with low magnification often rough or echinulate (e g *A flavus oryzae* group) The secondary thickenings in such conidiophores appear to have been laid down around protoplasmic areas, which for a time at least maintain contact with the primary wall outside The size and abundance of the pits in the mature conidiophore wall differ with the species, but in a general way correspond with the rate of withdrawal of the protoplasmic mass from its primitive connection with the original outer wall In some of the species in both groups warts, or superficial and usually more or less hemispherical concretions are found on the outer surfaces of the conidiophore wall, sometimes few and scattered widely, again fairly numerous but always unevenly distributed (e g *A ochraceus* group) These warts or concretions appear to be deposits of excreted substance possibly due to the evaporation of the numerous drops or globules of liquid abundantly visible upon the young and growing conidiophores

The color of the conidiophore wall may be homogeneous or the layers may differ markedly in shade In a number of the groups the entire wall is hyaline In certain other groups the outer layer is yellow as in *A ochraceus* and *A flavipes*, or it may be some shade of green brown, or avellaneous In some species the whole wall is colored for all or part of its length No explanation of these color differences is available, except possibly the varying concentration of aspergilline (Linossier, 1891) in the upper part of the conidiophores of members of the *A niger* group as well as in the conidial heads

The Vesicle

The conidiophore is usually much larger toward the apex than at the point of origin At the base of the head a further dilation occurs more or less abruptly to produce the vesicle (*blase* of the German mycologists) This vesicle is globose hemispherical elliptical or long clavate in various groups of the Aspergilli and furnishes an enlarged surface for the attachment of spore bearing cells The lumen of the vesicle is continuous with that of the upper part of the conidiophore a septum near the base of the head is occasionally but only rarely seen (see Corda *A mucoroides* for description) and has not been regularly found in any species

Sterigmata (Compare Fig 3)

The conidia bearing surface represented by the fertile area of the vesicle is closely covered by the simultaneous development of a layer of cells the sterigmata each in a general way perpendicular to a point on the fertile surface of the vesicle In figure 3A a single layer of such cells is shown each of which produces an unbranched chain of conidia In figure 3B each of the first series of cells or primary sterigmata bears two to several

cells the secondary sterigmata forming a crown or verticil at the apex Each of the secondary sterigmata bears one chain of conidia The mechanism shown in figure 3B would produce several times as many chains of conidia as figure 3A Such a head as figure 3B would be compact whereas figure 3A would represent a loose head

In figure 3A the cells in the single layer each producing a chain of spores are in the strict sense the sterigmata In figure 3B cells of the first layer or primary sterigmata produce verticils of cells the secondary sterigmata each of which is in the strict sense a sterigma The cells are usually characteristic in size and shape for series of closely related species Where there are both primary and secondary series the primary sterigmata are essentially supporting cells and vary much more in size and shape than do the secondary sterigmata For this reason they are more useful in species diagnosis than the secondary series

Various usages are found in the literature The primary sterigmata are often called *basidia* The secondary sterigmata are called *phialids* because they have somewhat the shape of the pharmacists phial (vial) In translating descriptions into the Latin Saccardo apparently followed the describers verbatim hence used no consistent terminology We find the primary sterigmata as sterigmata basidia or pseudobasidia and the secondary series as sterigmata pseudosterigmata ramuli (ramulis sporiferis) or even rami (branches) all of these usages have been homogenized here into primary and secondary sterigmata

Conidium Formation

The actual spore-producing cell or sterigma is definitely specialized It ordinarily consists of an essentially cylindrical body which after reaching a length more or less uniform for the species narrows into a spore-producing tube whose diameter is fairly uniform within the species Elongation is thenceforth confined to this spore-producing tube The nuclei in the sterigmata divide and one of each pair of daughter nuclei passes into the tube cell division follows Parallel with the repeated division of the sterigma nucleus the tube continues to elongate rapidly successively cutting off new sections and pushing the older cells outward Each such chain of spores typically consists then of series of equal sections cut from one tube or tip of a sterigma and each carries a daughter nucleus derived directly from the active nucleus of the sterigmatic cell at the base of the chain No further divisions occur among the cells in the chains Such chains often contain several hundreds of spores or conidia each of which is theoretically at least exactly like the rest hence fully capable of propagating the species (fig 6)

The Conidium, or Spore

The conidia are thus specialized propagative cells asexual in origin, produced by a complex cellular fruiting structure This consists (1) of a foot cell connected with the vegetative mycelium and usually imbedded in the moist substratum (2) of a conidiophore or stalk rising more or less vertically into the air to a distance typical of the species and enlarged at the apex to form the vesicle which is the central unit and (3) of a dilated head consisting of one or two series of cells, the outermost of which are specialized for the purpose of producing chains of cells (the conidia) each equally capable of carrying the genetic factors necessary to propagate the species

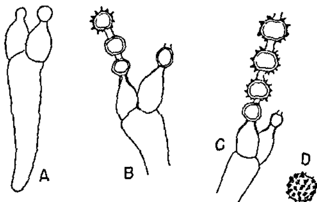


FIG 6 Camera lucida sketches showing progressive stages in conidium formation, × 700 *A* Initiation of conidium formation *B* Secondary sterigma bearing a chain of three conidia the outermost developing characteristic roughenings by the deposition of coloring matter between the outer (thin) and inner (firm) wall *C* Sterigma bearing a chain of conidia in which differentiation of the outermost spore is complete *D* A single mature conidium seen in surface view See discussion on page 24

After the conidium is separated by a septum from the mother cell or sterigma it remains so attached[1] as to draw nutrients from the parent cell at first while it assumes the size and shape characteristic of the species then it lays down within its original or primary wall a secondary cell wall whose color texture and marking are those of the species The secondary wall completely separates this spore from the parent cell (fig 6) Exact uniformity is not attained An occasional cell fails to develop some differences in size are usually evident markings while characteristic in nature and general pattern, are not always identical as to details For descriptive purposes size ranges are therefore more important than exact measurements and the nature of the markings found are more important than the relative

[1] Buller (Researches on Fungi V Chapter II 1933) discusses the primary septum as having a central pore through which connections are maintained

number and dimensions of such Such conidia may be very thin walled delicate and readily destroyed or firm walled almost impervious to stains and able to retain their vitality for many years They are extremely small light and float readily in air currents In many species the outer layer of the spore wall absorbs water slowly hence such spores tend to float in currents of fluid or to develop as mycelia covering the surfaces of liquid media Molds being typically aerobic normal colonies develop only on the surface of the substratum where oxygen is abundant and their spores can be discharged directly into the air Spores developing under submerged conditions in the absence of adequate oxygen produce fragmentary and defective mycelia only

The Connective

Descriptive literature often cites the presence of a connective or disjunctor , a bridge between conidia in the chain This is sometimes present again absent in the same microscopic preparation and when seen it appears as a short space between spores bridged by tran parent cell walls This is exactly what it is Cells cut off from a cylindrical tube may swell and assume subglobose form without breaking their area of contact or in the swelling and rounding up process they may partially or completely break that contact leaving the original cell wall of the tube, within which they developed as a bridge across the open space (fig 64 C) The critical examination of the developing cells in thousands of preparations have failed to justify interpretations which assume the degeneration of every alternate cell or fantastic fusions in the production of conidia The observation of connectives is therefore ordinarily worthless because morphologically it means nothing and it is not justified by successive studies of the same species

Endogenous Conidia

A spore is described as endogenous if it is formed within a tube or cell wall of a previously existing cell It may be extruded through a tube That tube may be used once only or many times The critical factor is the formation of a cell or spore within an existing specialized spore-bearing organ and its extrusion from that body through a fixed tube In Aspergillus the tip or tube of the sterigma elongates a cylindrical section is cut off carrying the tube wall and the septum at each end as the primary wall of the spore itself Within that primary wall the spore as an entity rounds itself up to characteristic form deposits or lays down its own wall with whatever coloration or markings may be typical of the species The primary wall may remain separate and distinct and in the ripe spore be visible under the microscope it may be blended with the secondary wall,

or it may not be determinable on the ripe conidium by ordinary examination In the sense of the definition above, no endogenous conidia appear in Aspergillus In cases the primary walls are seen to break away if ripe spores are mounted in fluid often carrying with them the granular materials which impart the characteristic marking to the spore hence leaving the wall of an *A niger* spore smooth (*A luteo-niger* Lutz)

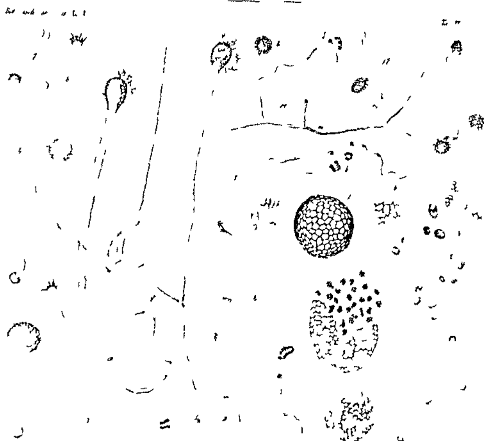


Fig 7 The development and relationship of *Aspergillus glaucus* and *Eurotium* after DeBary 1854

Perithecia

The general morphology of the perithecium of the *A glaucus* group (Eurotium) was described and figured by DeBary (1854 1870) while that of *A nidulans* was described by Eidam soon thereafter (1883) DeBary described the yellow to orange or ferrugineous perithecia as from 90 to 300 μ in diameter without ostiole and without specialized appendages (fig 7) These perithecia are borne above the surface of the substratum

and are never more than loosely hung in networks of hyphae they are often very abundant and dominate the color of the colony Perithecium formation is favored by abundance of assimilable carbohydrate but may or may not be completely suppressed by its replacement with nitrogenous products transfers from the same culture have developed colonies yellow from perithecia when grown on sugar solution or dense green without sign of perithecia upon a peptone medium or a piece of leather

The perithecium of Aspergillus arises from a branch which coils in various manners in the different species to become the ascogone as figured by Dangeard from forms reported as *F herbariorum* *A flavus* *A fumigatus* *S ochracea* *S nidulans* by Fraser and Chambers for *A herbariorum* and by Dale (1909) for *A repens* No fertilization process was demonstrated in those studies Dangeard (1907) reported the cells in the fruiting apparatus as multinucleate in *F herbariorum* but to be mononucleate in the other species figured In general however the development of an ascogone followed by the development of the perithecial wall and accessory cell masses from vegetative hyphae below the ascogone has been roughly described and figured for the group represented by *A glaucus* and *A nidulans* but interpretation of the other specific names used by Dangeard is doubtful even for grouping on the basis of the descriptions and figures given

Henrard (1934) working with 15 species of Aspergilli reports that all of them are sexually homothallic He summarized the literature by saying that homothallism in the Aspergilli has been affirmed by Kniep (1928) for *A repens* by Schwartz (1928) for *A ruber* *A repens* four other strains of *A glaucus* and four strains of *A nidulans* by Blochwitz (1932) for six strains in the *A glaucus* group and by Greene (1933) for *A fischeri*

So far then as present information goes heterothallism cannot be assumed to account for the great variability of the Aspergilli

Perithecia are regularly found in most of the species of the *A glaucus* group and in *A fischeri* which is closely related to *A fumigatus* and in a series of closely related forms in the *A nidulans* group They are not sporadic or dependent upon unknown conditions but are regularly produced in media which are adequately supplied with sugars and the salts in routine use Their presence in *A wentii* and *A citrisporus* was asserted by Thaxter (personal communication) without producing either material or description of the ascospores Dangeard (1907) claimed to have found ascospores within the sclerotia of *A niger* but failed to describe them Diligent search over many years has failed thus far to confirm either statement Still it may be assumed that such sclerotia may be the homologue of structures which under some conditions might become perithecia

The Ascospore

So far as fully described, the ascospore of Aspergillus follows the general type shown in the figures and description of DeBary. In the course of its development the secondary thickening of the cell wall develops in the form of two symmetrical valves suggesting the arrangement found in the shell of a bivalve mollusk, such as the hard clam (*Venus mercenaria*). The ripe ascospore is commonly shaped as a double convex lens with the valves more or less closely in contact at the edges. A series of variations upon this basic pattern occur and characterize particular species (figs. 27, 34, and 43). If the exospore is smooth and the margin of the valves is not marked by folds or ridges figure 27A characteristic of *A. repens* appears; if the exospore is rough in the absence of marginal folds figure 27D characteristic of *A. amstelodami* results; if the exospore is rough and the margin of the valves bear folds or ridges figure 43C characteristic of *A. rugulosus* and *A. fischeri* is produced. An extreme development of the marginal folds is seen in *A. variecolor* figure 43D.

When such an ascospore germinates (fig. 8A), the figure first shown by DeBary (1854) develops. Exactly the same type of germination is shown by *A. nidulans* in which as the spore swells the valves first separate at one edge, then parting completely, remain on opposite sides of the germinated spore conspicuously identifiable by the bright purple red color of the valves (Thom and Church, 1926).

Hulle Cells

Mature perithecia were described for *A. nidulans* by Eidam (1883) but without giving attention to their origin. In his description Eidam pictured a loose network of hyphae more or less completely surrounding the perithecium containing large numbers of "Hulle" cells, terminal or intercalary cells which swell and become vesiculose, elliptical or almost globose, then develop very heavy thick walls almost obliterating the cell lumen (fig. 49). These cells were later noted by Dangeard (1907) who designated them as chlamydospores but failed to present evidence of function as propagative cells. These cells are abundant in connection with perithecia in all strains of the *A. nidulans* group but are lacking in *A. unguis* which does not produce perithecia. Cells of the same general character appear in sterile masses of hypae in strains of the *A. ustus* and *A. flavipes* groups (fig. 49) and in some strains of the *A. versicolor* group while thick walled septate hyphae at least suggestive of hulle cells appear in *Aspergillus carneus* in the *A. terreus* group (fig. 49F).

In the *A. nidulans* group hulle cells, Eidamsche blasen or chlamydospore (terms found in the literature) are always produced in connection with perithecium formation (fig. 42). In the *A. flavipes*, *A. ustus* and *A.*

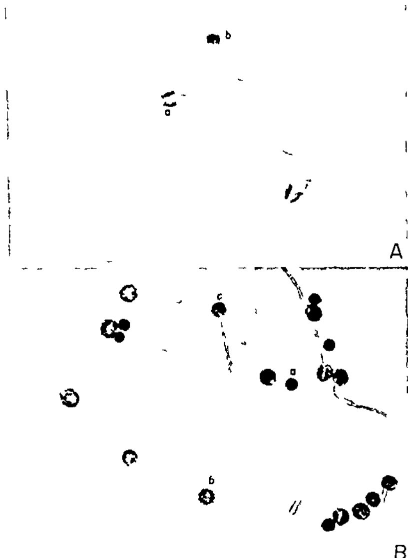


FIG 8 Spore germination *A* Germinated ascospores of *Aspergillus nidulans*, × 750 *a* ascospore in profile showing how the two valves comprising the spore wall are pushed apart as the spore content enlarges and the sporeling develops *b* the same in face view *B* Germinating conidia of *Aspergillus niger* × 600 *a* ungerminated spore *b* spore in early stage of germination *c* germinated spore showing rapidly elongating sporeling

versicolor groups, they occur in scattered aggregates of varying conspicuousness, although no perithecia have been found Occasionally, as in one of the *A flavipes* group, foot cells and sterile cells which appear to be *conidiophores ending* in points instead of heads appear These aborted conidiophores vary from nearly full length to vestigial From the study of such material it is believed that Eidam s hulle cells represent aborted conidiophores surrounding the perithecia of *A nidulans* and they may be indicative at least of a vestigial precursor of perithecium formation when they occur elsewhere

Hulle cells do not, however, accompany the perithecia of either the *A glaucus* group or *A fischeri* In the former the perithecia are smooth walled and naked, in the latter, they are surrounded by a loose network of sterile but unspecialized hyphae

In *A panamensis* (Raper and Thom 1944) aborted conidiophores commonly occur, but no hulle cells have yet been observed In *A unguis* (Thom and Raper, 1939), which is one of the *A nidulans* group lacking only the ascosporic phase the long sterile thick walled spears suggest homologous structures To summarize, hulle cells are specialized structures that normally occur in certain groups of the Aspergilli They are of somewhat questionable origin and in our experience are not known to serve any functional purpose Schwartz (1928) figures the hulle cells of *A nidulans* as capable of germinating and thus acting as reproductive cells Since the cells of different groups are fairly characteristic, they often provide valuable diagnostic group and species characters

Sclerotia

Definitely hard masses with characteristic surface marking and coloration and consisting of thick walled parenchyma like cells occur in several groups of Aspergilli Such structures have not been seen in the groups typified by *A clavatus A glaucus, A fumigatus A nidulans A ustus A versicolor*, or *A terreus* On the other hand they develop regularly in certain members of the *A candidus A niger, A wentii, A tamarii A flavus-oryzae* and in the *A orchaceus* groups in other members of these same groups grown under similar conditions no *such structures have* been found In occasional strains of *A flavipes* dark hyphal masses are seen but these are not sufficiently compact to be considered true sclerotia *The development of sclerotia has not been followed in sufficient detail in* any species to fix their genetic significance but the specialized characteristic structure of the sclerotia of many species lends color to the report of *ascospore formation by Dangeard (1907) although no one else seems to* have been able to verify such development Environmental factors are known to influence sclerotium development but these have not been *carefully analyzed*

Chapter IV

CULTIVATION AND EXAMINATION

An Aspergillus occurring upon a natural substratum may frequently be identified as to group from the original material if it belongs to one of the large and well marked groups Among members of the same group however differences in the nature and composition of the substratum produce marked contrasts in colony appearance quantity of growth coloration measurements of fruiting structures and in the appearance of conidiophores and conidial masses Many Aspergilli can be identified as to group from dried herbarium materials but the process of drying generally changes the colors materially and renders the hyphal masses so fragile that the morphological details necessary for identification inside a particular group are often obliterated or made difficult to interpret Although accurate placing of such materials is sometimes possible, a much more satisfactory identification may be reached by transferring fresh material to culture media of known composition followed by purification of the cultures so that they present single species or strains for intensive study

Two types of material for identification are regularly encountered by one undertaking a study of the Aspergilli (1) the culture already isolated by another worker and (2) the moldy substratum with its natural flora of micro-organisms often representing several or many species including miscellaneous molds bacteria actinomycetes and even protozoa and other forms In either case the final decision as to the proper name of an Aspergillus must usually be sought in fresh cultures made by the one responsible for identification

Classification within the genus has become so dependent upon observation in pure culture that the whole subject of laboratory cultivation including favorable substrata culture making and culture handling needs to be considered

CULTURE MEDIA

Pure culture upon known substrata is almost essential to the identification of Aspergilli Since the morphological responses to diverse nutrients and especially to the stimulus of mixtures of other molds and bacteria growing with any particular species is great the study of each strain or species in pure culture in media of known composition is practically necessary

Aside from Raulin s group in Paris most of the early culture work with Aspergilli was carried through upon so-called natural media DeBary and

Brefeld used decoctions of horse dung variously diluted and stiffened with gelatin. Much of the European work was done with brewery wort, Mazé (personal communication 1904) used extract of white beans, potato and carrot decoctions have been widely used. Bainier grew his molds and described many of them upon sticks of licorice root, others preferred plugs of potato or of carrot, string beans, etc. The primary aim was to obtain an optimum growth of the mold under observation, rather than to analyze its relation to the substratum or furnish comparative data to distinguish it from other members of a series.

An optimum culture substratum for comparative study of the Aspergilli needs to contain the necessary chemical elements in the form of pure but assimilable salts supplemented by carbohydrates of such structure as to be available to the largest number of species, and purchasable in pure form in the chemical trade. Supplementary and often very important information must be sought from variations in the proportions of nutrients used or in the introduction of widely different substances in replacement of particular components of media already used. The Aspergilli as they are isolated from nature are not very dependent upon vitamins or growth promoting substances. For cultivation upon solid substrata agar is almost universally employed as a gelling agent.

When the study of a large number of molds is undertaken comparison of these molds under controlled and reproducible conditions of growth become essential. Foremost among the conditions which must be standardized is the culture medium or substratum. Various authors have proposed standardized and reproducible formulae which in their experience have provided uniform cultures over long periods, hence are of value for comparative studies. A series of such media are presented.

Czapek's Solution Agar

As a routine medium for comparative work the following formula originally adapted from Czapek (1902, 1903) by Dox (1910) has been widely used. Minor variations in quantities apparently do not affect the reactions. *Cultural information given in this manual is obtained from growth upon media produced by this formula unless otherwise specified.*

Czapek's solution agar

Water	1 000 cc
$NaNO_3$	3 0 grams
K_2HPO_4	1 0 gram
$MgSO_4 \cdot 7H_2O$	0 5 gram
KCl	0 5 gram
$FeSO_4 \cdot 7H_2O$	0 01 gram
Sucrose (Cube or other good commercial grade)	30 0 grams
Agar	15 0 grams

To reduce caramelization the sugar is added just prior to final sterilization

Czapek's solution agar is not offered as an optimum substratum for any particular species but as a mixture approximately neutral in reaction which is readily made in any laboratory in fairly uniform manner, and which permits moderately vigorous growth of nearly all of the saprophytic Aspergilli The quantities of mycelium and conidia produced by many forms are much greater upon other media but for comparative study a moderate growth of the majority of the species is more useful than the great mass of mycelium and conidia which are readily obtained by using enriched substrata

Since the purpose of the Czapek formula is to insure the presence of the chemical elements required in quantities sufficient to support good growth it is frequently modified as to quantities and nutrients introduced For some Aspergilli such as the *A glaucus* group the addition of 20 percent or even 40 percent of sucrose has proved useful Ammonium nitrate is sometimes substituted for sodium nitrate but with the loss of information as to whether the mold utilizes the ammonia or the nitrate or both Dextrose is commonly substituted for sucrose The monobasic potassium phosphate prevents precipitation of certain components in sterilization but it produces an acid instead of a neutral medium hence complicates many pieces of work The introduction of peptone or yeast extract increases the sporulation of some forms These and other changes are made however by investigators who still refer to Czapek's solution as the basis of their work

Biourge in his monograph of Penicillium (1923) and in his unpublished Manuscript of Aspergillus (1939), put much emphasis upon the method of preparing the neutral Raulin medium which he used for the growth of the colonies analyzed in making his species diagnoses

Neutral Raulin's Solution—Dierckx Biourge

1 Magnesium carbonate 0 40 gram
Tartaric acid 0 71 gram
Triturate in a mortar with a few drops of distilled water and add quickly to a flask of distilled water make up to 100 ml

2 To a liter flask with 800 to 900 ml distilled water add

Sucrose	46 60 grams
Ammonium nitrate	2 66 grams
Ammonium phosphate	0 40 gram
Potassium carbonate	0 40 gram
Ammonium sulphate	0 16 gram
Zinc sulphate	0 04 gram
Iron sulphate	0 04 gram

3 Add 66 to 67 ml of the magnesium tartrate solution (1) to the mineral salt sucrose solution (2) and make up to 1 000 ml with distilled water

In his detailed study of the *A niger* group, Biourge's pupil, Mosseray (1934a) gives his simplified Raulin's solution as follows

Water distilled	1,000 ml
Sucrose	50 grams
Tartaric acid[1]	0.40 gram
Magnesium carbonate[1]	0.250 gram
Ammonium nitrate[2]	0.250 gram
Potassium carbonate[2]	0.40 gram
Ammonium phosphate $(NH_4)_2HPO_4$[1]	0.40 gram
Ammonium sulphate[1]	0.20 gram
Iron sulphate[1] (cryst)	0.05 gram
Zinc sulphate[1] (cryst)	0.05 gram
Agar agar[1]	20.00 grams

Sterilize 120° C for 20 minutes

Steinberg's Solution

Steinberg in the course of many years' investigation of a single strain of *Aspergillus niger* (NRRL No 334 Thom No 4247) developed a basic formula for testing other phases of the nutrition of his mold It is called the "dibasic optimum" solution and carries mannitol instead of sucrose sodium nitrite instead of ammonium nitrate, with the addition of sufficient sulphuric acid to obtain any desired reaction Interpolations into this solution offer many possibilities

Steinberg's dibasic optimum (Thom and Steinberg 1939)

Water (distilled in Pyrex still)	1 000.0 grams
d Mannitol	50.0 grams
NaNO	2.0 grams
K_2HPO_4	0.35 gram
$MgSO_4 \cdot 7H_2O$	0.25 gram
$FeSO_4 \cdot 7H_2O$	0.001 gram
$ZnSO_4 \cdot 7H_2O$	0.00088 gram
$CuSO_4 \cdot 5H_2O$	0.00020 gram
$MnSO_4 \cdot 4H_2O$	0.00012 gram
$NaMoO_4 \cdot 2H_2O$	0.00005 gram
H_2SO_4 to pH 4	

A number of so-called natural substrata including malt extract and hay infusion agars are very useful in the study of the Aspergilli A great majority of species sporulate more freely upon malt extract than upon Czapek's solution agar (fig 9), and for this reason it is very useful where large quantities of spores are desired This medium is however less diagnostic than Czapek's solution

[1] Merck reagents
[2] Kahlbaum reagents

Malt Extract Agar (Blakeslee s Formula 1915)

Distilled water	1 000 cc
Malt extract	20 grams
Peptone	1 gram
Dextrose	20 grams
Agar	20 grams

Add dextrose just prior to final sterilization Conidial structures are generally more numerous and are often borne on shorter conidiophores and there is an almost complete absence of coloration in the substratum Except in a few isolated cases coloration of the spore heads themselves is not materially altered While the production of exudate in the form of drops is not characteristic of many of the Aspergilli it can be generally said that droplet formation on malt agar is much less than upon Czapek s solution agar

Hay infusion agar is very useful in the isolation of Aspergilli from nature A 1 10 suspension of soil in sterile water is streaked on hay infusion agar plates and incubated for one week to 10 days Isolations are then made from individual fruiting structures with the aid of a low power binocular

Hay Infusion Agar

Distilled water	1 000 cc
Decomposing hay	50 grams
Autoclave for 30 minutes at 15 pounds Filter	
Infusion filtrate	1 000 cc
K_2HPO_4	2 grams
Agar	15 grams
Adjust pH to 6.2±	

No Aspergillus makes a luxuriant growth upon this medium but a great variety of forms make a limited development Furthermore such fruiting structures as are produced are generally characteristic of the different species present It thus constitutes a very favorable substratum with which to analyze and isolate the Aspergilli occurring in soils or other natural substrates The medium is likewise useful for securing limited sporulation of certain forms such as *Aspergillus sparsus* which fruit very sparsely upon Czapek s solution agar

Czapek s solution agar enriched with peptone or corn steeping liquor is often very useful For example in the cultivation of members of the glaucus group the addition of a limited amount of peptone greatly increases the production of conidia while addition of a small amount of corn steeping liquor (e g 0 2 percent) increases the growth of most forms without markedly affecting the character of the resulting colonies

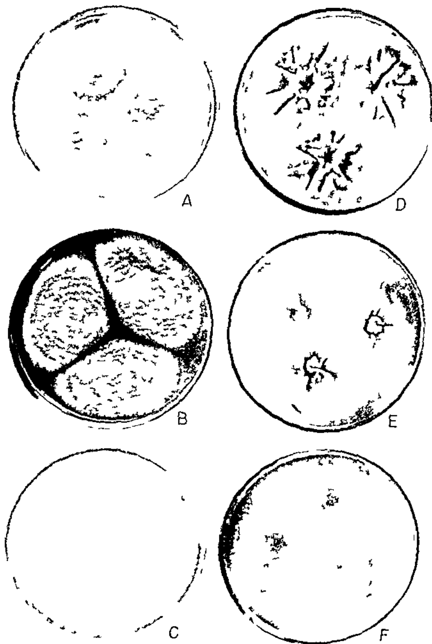


Fig 9 Influence of substratum *A–C Aspergillus variecolor* NRRL No 212 growing upon Czapek s solution malt extract and hay infusion agars respectively 10 days room temperature Note particularly the heavy development of perithecia upon malt and the very sparse development of the same upon hay infusion agar *D–F Aspergillus caespitosus* NRRL No 1929 growing upon the same media under similar conditions Upon Czapek s solution agar (*D*) dark hulle cell masses develop along with a limited development of conidial structures upon malt (*E*) and hay infusion (*F*) agars hulle cell masses are lacking and conidial structures are very abundant

SPORULATION MEDIA

In addition to the ordinary handling of molds in the laboratory exploration of their biochemical potentialities often necessitates the production of considerable quantities of spores. Whereas some of the media listed above may be employed for this purpose, it is generally advisable to employ so-called sporulation media. Requirements of different species and strains vary and one frequently has to develop special solutions to meet the needs of particular organisms under study. Such media may be used either as liquid substrata or as solutions solidified with agar. In either case the objective is the same: to secure the maximum production of spores with the production of as little vegetative mycelium as possible.[1]

A number of sporulation media for use with different molds have been developed by Dr. A. J. Moyer. Three of these will be cited, while additional formulae may be found in the papers published by members of the Fermentation Division, Northern Regional Research Laboratory.

Sporulation medium for A. niger (Moyer, Wells, Stubbs, Herrick, and May, 1937)

Glucose	91.3 grams
NH_4NO_3	0.450 gram
KH_2PO_4	0.0[illegible] gram
$MgSO_4 \cdot 7H_2O$	0.060 gram
Beer	60.0 ml
Distilled water to make	1 liter

This solution can also be used as a solid medium by the addition of 0.5 gm. per liter $CaCO_3$ and 30.0 gm. per liter agar. The above solution was subsequently modified as follows (Gastrock, Porges, Wells, and Moyer, 1938):

Glucose	50.0 grams
$(NH_4)_2HPO_4$	0.560 gram
KH_2PO_4	0.144 gram
$MgSO_4 \cdot 7H_2O$	0.1[illegible]0 gram
Peptone	0.[illegible]0 gram
Beer	45.0 ml
Distilled water to make	1 liter

[1] For the surface inoculation of nutrient solutions in small flasks or other containers, dry spores in quantity can be removed from agar surfaces and floated on the liquid surface by means of a 5 mm. loop. To inoculate from a liquid culture, the usual procedure is to remove a small portion of the heavily sporing mat, transfer this to the solution, and dislodge the spores by vigorous agitation. Spores from either type of culture can be suspended in water and used as inoculum in submerged or shaken cultures. The addition of sodium lauryl sulfonate to the suspending water in a concentration of 1:10,000 aids greatly in securing uniform spore suspensions.

Sporulation medium for A flavus (Moyer, Personal communication)

Glucose (commercial)	165 0 grams
Bacto Peptone	1 0 gram
$MgSO_4$ $7H_2O$	0 050 gram
KH_2PO_4	0 060 gram
KNO_3	0 500 gram
Fe (as tartrate)	0 040 gram
Agar	0 25 gram
Distilled water to make	1 liter

The above solution can be used as a solid medium by increasing the agar concentration to 30 0 gm per liter (The small amount of agar included in the basic formula is added solely for the purpose of increasing viscosity) Incubation should be at 22° to 24° C This factor is critical for maximum spore production with the kojic acid producing strain, NRRL No 484 (Thom No 3538) for which the medium was developed This medium can also be successfully employed for spore production in *A niger* by incubating cultures at 30° C

Abundant sporulation of many strains and species can be secured by cultivation upon bread whole cereal grains, or various types of milled products of the same Bread, if used, should not contain proprionates or other mold inhibitors The material to be inoculated should be moist but in no sense wet, and special precautions must be taken to insure that the grain or bread is properly sterilized before being used The use of grain as a substratum for molds dates back to prehistoric time in the fermentation industries of the Orient where rice was and still is, commonly used to produce the koji," or inoculum used in the alcoholic and soya fermentation industries of that area In its classic usage, the molds cultivated were mostly members of the *A flavus oryzae* group but experience has shown that this general type of medium can be used to advantage to secure heavy sporulation of many other forms From such material series of surface fermentation flasks or other vessels can be uniformly inoculated by various means involving aspiration of spores or the direct transfer of heavily spore laden particles Spore suspensions can be prepared and used for seeding submerged cultures

TYPES OF CULTURE

Cultures for grouping and identification of the Aspergilli should be grown in petri dishes At the same time an adequate number of slanted tubes should be inoculated and held in reserve as an uncontaminated stock culture Colonies so situated in the petri dish that they can be viewed directly under low magnifications with the compound microscope are necessary to supply a clear picture of the structure and course of development of mycelium and fruiting parts Such colonies can be obtained by several

procedures some of which have been developed for special tests or for individual mold problems Practices useful in bacteriology are often applicable to the problems of the mycologist Selection of a procedure which satisfactorily provides the information necessary to describe an Aspergillus calls for discussion of a series of procedures commonly in use Only in that way may we show why some of these are adapted to the problems of identifying an Aspergillus while others fail for specified reasons, to furnish important observations

Spot Inoculations

Over long periods and in the hands of many investigators some type of mass conidial inoculum has given dependable and reproducible results The most common method of transfer and on the whole probably the most satisfactory one for maintaining a strain of Aspergillus as well as other molds is the removal from the stock culture of a variable mass of conidia fruiting structures or vegetative hyphae with some sort of needle or loop and the transfer of this material to selected positions on fresh medium Practices differ With the Aspergilli colonies so placed as to permit radiate development from the point of inoculation are most satisfactory for study Where there are two or three colonies to the plate these eventually reach into each other s zones of influence They may blend and become indistinct or inhibit each other and leave sterile bands between the colonies Both types of culture furnish useful data Usually, the line where two colonies approach and partially or completely inhibit each other hastens fruiting in the adjacent margins and permits favorable examination with the compound microscope The opposite margin of the same colony unaffected by competition furnishes at the same time the normal and symmetrical growth which is typical of the species The one-colony plate usually provides the most striking exhibit of the species but the 2 or 3-colony plate is the most generally useful (fig 10)

Once an Aspergillus has been obtained in pure culture the most effective way to insure that plates will contain 1 2 or 3 colonies as desired is to suspend a quantity of spores in melted agar at approximately 45 C allow this to solidify and then transfer small quantities of the gelled suspension to the surface of plates or agar tubes in the positions where the colonies are desired With the Aspergilli as with all of the molds which produce dry spores it is often very difficult to secure placement of colonies at selected positions and only at such positions without the use of some type of wetted inoculum

In the routine examination of Aspergilli where it is not essential that the number of colonies be limited to 3 or less satisfactory transfers have been made by using a sharp nichrome wire as an inoculating needle and selecting

the inoculum from a colony in a petri dish by working under a 10× pocket magnifier, or under a binocular microscope of the Greenough type carrying similarly magnifying lenses Material to be removed can be exactly located in the parent colony It is found possible in dealing with most Aspergilli (1) to remove conidia from a single head, (2) to remove one or more heads borne upon a single hypha at the margin of the colony, or (3) to select vegetative hyphal tips from a single mycelial sector It is usually possible to avoid (1) heads and conidia of other species or strains (2) foreign mycelia and (3) bacterial contaminations present in the substratum Purity in repeated culture over many years has been possible by this procedure

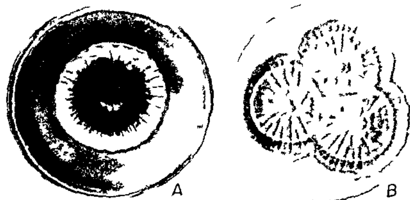


Fig 10 Single and three point cultures of *Aspergillus foetidus* on Czapek s solution agar room temperature 10 days × ½ approximately Note that spore production extends to the colony margins in the central triangle of figure *B* and almost to the outer colony margins as well In figure *A* sporulation is limited to the central area only Figure *B* is favorable for direct examination with the low power objective of the compound microscope figure *A* is not

Dilution Cultures

If dilution cultures are to be used the suspension of conidia should be sufficiently dilute so that the individual petri dish will show not more than six but preferably not over three colonies Such dilutions are difficult to gauge and often result in some plates crowded with numerous colonies while others contain none The colony locations are not under control In general, the dilution of spores in successive water blanks with the subsequent plating of aliquots from such dilutions has not been found satisfactory

An alternate technique involves the introduction of one loopful of inoculating material into a tube of melted agar which is then rolled or shaken

and a loopful removed to a second tube followed by the same manipulation a third or fourth time The higher dilutions are then poured into petri dishes where colonies develop This method has all the faults of the water blank dilution technique The procedure has been widely used but has not been followed in our study of the Aspergilli Blakeslee (1915) and other mycologists have prepared such dilutions in bottles by slowly rotating the bottle in a cold water or ice bath, thus allowing the agar to congeal in a thin layer against the glass surface This practice has little to recommend it (1) the resulting colonies could be isolated more readily from a petri dish, and (2) it is basically impractical to make satisfactory observations regarding the character and structure of a mold colony or the gross features of its fruiting structures when observations have to be made through the curved and non uniform walls of a glass bottle

Smears and Streak Cultures

The practice of smearing a suspension of mold spores over the whole plate or the whole length of a slanted agar tube results in a growth of mycelium which covers the entire surface and usually produces a greater mass of conidia But individual colonies are usually unidentifiable in such preparations hence they are of little value in providing those critical details of colony habit coloration and texture necessary for identification and classification In general mycelium derived from different conidia of the same strain will intertwine without inhibition and even anastomose if they come into contact in the early stages of growth for example before any sign of conidium production appears If the spacing of the spores is great enough to permit the establishment of small fruiting colonies before such contact is made the colonies frequently do not converge and complete coverage of the surface of the agar with the production of maximal quantities of conidia does not occur Closely placed seeding of spores is useful to obtain the largest possible supply of conidia but to study the normal characters of a species individual colonies must be allowed to develop without interference by others of the same or different species

Streak cultures can be employed to advantage in freeing one mold from another or from other contaminating organisms By touching a sterile moistened needle to a single conidial head or other selected fruiting surface of limited extent and subsequently streaking this repeatedly across the surface of an agar plate isolated colonies of the desired species can usually be obtained Occasionally it is desirable to streak two plates in succession in order to secure a satisfactory separation of colonies In any case the spore source should be selected with great care and the use of a low power binocular microscope or pocket magnifier is recommended When this method is employed it is desirable to reisolate from these individual colo-

nies as soon as possible after sporulation begins For freeing one species or strain of *Aspergillus from another*, or from a contaminating Penicillium, streaking upon Czapek's solution agar usually gives satisfactory results The method finds its greatest usefulness in separating comparatively slow growing molds, such as the Aspergilli, from such very rapidly growing forms as the Mucoraceae, *Trichoderma*, etc The critical step in this procedure is to isolate the slow growing Aspergilli during the first two to three days before the whole plate is overrun by the spreading faster growing forms If the contamination is bacterial in nature malt extract agar or some other medium of more acid reaction should be substituted Diluting the spores in water before streaking is not recommended since this often tends to disperse the contaminating organism more than it does the desired form, this is especially true with bacterial contaminations actinomycetes, and other minute forms

Single Spore Cultures

The isolation of cultures from single spores is a time-honored technique with many mycologists and with many workers it is considered a "must" When properly employed, there is much to recommend this practice There are also certain dangers inherent in this procedure hence we believe it worth while to consider the subject at some length and to analyze both its advantages and its limitations As generally employed, the primary objective of single spore isolations is to secure cultures of unquestionable purity If the operation is skillfully performed and adequately verified there can be no doubt but that the resulting colony will represent a pure culture of a single species or strain The continued purity and physiological stability of such cultures however cannot be taken for granted There is no substitute for vigilance, and the culture thus isolated must be kept under critical observation

As a means of purifying a culture i e , separating it from foreign forms, the single spore method undoubtedly has its place but it cannot be recommended as a means of preserving the morphological and physiological stability of a particular strain One has only to study the reports of Stakman and his associates to realize that monospore selection and isolation is not a touchstone to strain stability In fact they have ably exploited the technique to show just the opposite While we do not have an accumulation of data on the Aspergilli and related genera which can compare with that on the smuts and other pathogenic fungi we do have enough information to know that one of the best methods of obtaining change in some of the Aspergilli is to make a series of successive monosporous isolations For every species and strain there is apparently a normal range of natural variation and by isolating single spores it is often possible to secure certain

progeny which represent essentially the outside limits of such variation This is worth while since it offers one means of securing strains which may prove more useful than the parent culture for some particular purpose, industrial or otherwise The single spore method can be of real value in purifying a culture It can be of real value as a means of dissecting a culture But once a promising strain has been discovered and its purity is established perpetuation by the transfer of masses of conidia is the best safe-guard for preserving in a constant condition, its morphological and physiological characteristics

Some of the techniques by which such single spore isolations can be made follow

(1) *Serial Dilution* Spores are thoroughly suspended in water and the resulting suspension is subsequently diluted in sterile water blanks in steps of 1 5 or 1 10 One cubic centimeter aliquots from two or three selected dilutions depending upon the density of the original suspension are added to sterile petri dishes Melted agar at approximately 45 C is then added to these plates which are rotated to secure uniform mixing of the still liquid agar and the diluted spore suspension The plates are then incubated at room temperature and isolates are made from plates showing a limited number of colonies which are uniformly separated Using this technique one cannot be certain that any particular colony results from a single spore but if the original suspension was properly prepared one can feel sure that more then 95 percent of the colonies resulted from single spores More uniform suspensions of spores can be obtained by adding to the suspending medium some suitable detergent or aerosol For this purpose we have successfully used sodium lauryl sulfonate in concentrations of 1 10 000 or 1 100 000 without apparent harmful effect upon the molds under study The dilution method of securing single spore isolations is more rapid than any other and for many purposes it is quite satisfactory

(2) *Selection and Removal of Individual Spores* Where the investigator wishes to be positive that every colony results from a single spore it is necessary to employ some technique combining actual microscopic examination with some device for the mechanical removal of selected spores The various types of micro-manipulators are well suited for this work and with sufficient practice single spore isolations can be made quite satisfactorily with any of these Dilute spore suspensions in water are mounted on the undersurface of a cover slip supported by a glass chamber open at either one or both ends With the aid of mechanical controls and a micro-pipette a single spore is withdrawn from the suspension and ejected upon a suitable substratum where the spore develops into a mature colony

Single spores can likewise be removed by mechanical cutting devices such as those described by LaRue (1920) Keitt (1915) and Lambert (1939)

A comparatively thin spore suspension is spread on an agar surface and single well-separated spores are located with the microscope A small cutting device, mounted on a holder screwed into the nose-piece of the same microscope is lowered into the agar and a small block bearing the selected spore is removed This is subsequently transferred to a suitable culture surface where the colony develops

At the Northern Regional Research Laboratory we have employed a somewhat different and simpler method A thin spore suspension is spread evenly over the surface of a firm agar gel that has been specially filtered to remove all particulate matter This is incubated overnight and the spores allowed to germinate On the following day well separated sporelings are



FIG 11 Single spore isolation A A single well isolated germinated conidium of *Aspergillus niger* B The same removed on a small agar block and transplanted to a fresh agar plate as described in the text p 44 × 300

located with the aid of a microscope and their positions marked on the under surface of the culture dish These are then checked with a 8 mm objective and 10× or 15× oculars to insure that there are no other un germinated spores in the same area Using a wide-field binocular of the Greenough type and very small micro scalpels fashioned out of platinum iridium wire (B and S gauge 22 or 24) small agar blocks on which the spores spores rest are transplanted to fresh agar plates Each of these agar blocks is then examined again with the 8 mm objective to insure that the selected spore has been transplanted An experienced worker can isolate from 25 to 30 spores within a period of an hour Photographs showing essential steps in this technique are presented in figure 11

Hanging Drop Cultures

The drop of culture fluid inoculated with conidia and hanging from a slide or cover glass into a closed chamber can be incubated and examined readily It furnishes information as to the percentage of conidia which are viable, the changes which occur in the germinating spore such as swelling bursting along definite lines germination from specialized germ pores or branching of the germ tube but descriptions of fruiting structures in such hanging drops are worthless in the study of the mold colony or normal fruiting habits of the species

TEMPERATURE

The great majority of Aspergilli grow well and sporulate abundantly at temperatures of 23 to 26 C For this reason most cultures can be incubated on laboratory tables or shelves and it is not necessary to give special consideration to incubation There are however certain exceptions The large- spored members of the A. pergillus glaucus group such as *A echinulatus* and *A niveo-glaucus* grow more rapidly and fruit more abundantly at 20 than at 24 to 25 C (Thom and Raper 1941) This temperature response is especially marked in *A medius* at 18 to 20 C growth is rapid abundant large conidial heads are produced and numerous perithecia are developed at 25 C and above growth is restricted few and smaller conidial heads are developed and only occasionally perithecia are produced (fig 12) On the other hand the very abundant small spored members of the *A glaucus* group such as *A repens A chevalieri* and *A amstelodami* grow rapidly and fruit abundantly at 30 C (Thom and Raper 1941) In *A janus* (Raper and Thom 1944) two different types of conidial heads are produced and the ratio of these types is strongly influenced by temperature (fig 12) at 18 to 20 C almost all heads are white with clavate vesicles and are borne upon long conidiophores at 30 C almost all heads are dark green with globose vesicles and are borne upon short conidiophores (see species description p 187)

When grown upon suitable media and incubated at 20° C in the presence of light or alternate light and darkness *Aspergillus giganteus* produces large heads on long conidiophores ranging up to 5 or even 10 cm In similar cultures incubated at 30 C heads are smaller and conidiophores are uniformly short rarely exceeding 1 cm in length whether the cultures are exposed to light or incubated in total darkness *A terreus A carneus* and *A fischeri* thrive at temperatures up to 35 C while *A fumigatus* grows well at 45 or even 50 C In all of these forms growth is more rapid and sporulation more abundant at 30 C than at normal laboratory tempera

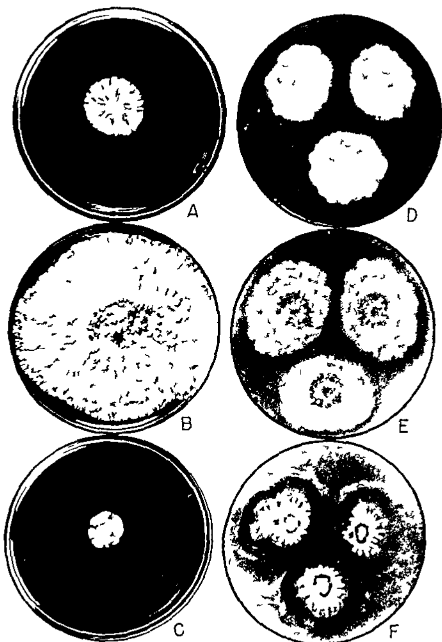


FIG 12 Influence of temperature upon growth and sporulation in *Aspergillus medius* NRRL No 124 and *Aspergillus janus* NRRL No 1787 *A B* and *C Aspergillus medius* 17 days at 12 C 20 to 22 C and 30 C respectively note maximum growth and limited sporulation at 20 to 22 C *D E* and *F Aspergillus janus* 3 weeks at 20 C 24 to 25 C and 30 C respectively note evidence of white heads only at 20 C abundance of white and presence of green heads at 24 to 25 C and green heads only at 30 C Temperature is the only variable

tures of 24° to 25° C, although wholly typical cultures are produced at both temperature levels

In the production of spores for biochemical investigation (see p 37) and in the conduct of such studies themselves temperature is often very important In work of this kind it is essential to determine the optimum temperature for each organism and process, and then to control this within a narrow range in order to secure consistent and reproducible results

Temperature is known to be a critical or limiting factor only in a few species but its effect in these is sufficiently pronounced that its influence should not be disregarded in any case

CULTURE TOOLS AND EQUIPMENT

Transfer Needles

In making transfers we have used with satisfaction for many years a No 20 or No 22 B and S gauge nichrome wire thrust into a slender brass or stainless steel tube so that only about 15 to 20 mm are exposed (The tubing employed is of the type used in temperature controls of mechanical refrigerators and other thermostatically controlled devices) The point of this wire is then ground down to the sharpness and smoothness of a needle This instrument can be heated to redness in the flame a great many times with only the occasional necessity of resharpening It thus has many advantages beside cheapness it is firmer and takes a better point than platinum it withstands sterilization in the flame which promptly destroys the usefulness of steel and it can be made any size or length to suit the workman s purposes or preferences

Loops

Loops of various dimensions are very useful and can be inserted into handles made from small brass or stainless steel tubing as noted above These too can be fashioned from nichrome wire but it has been our experience that loops made of platinum iridium wire posse s certain marked advantages While these are not as rigid as nichrome loops they are much more rigid than loops of pure platinum and they can be re-heated indefinitely without corroding Loops of this type will be found especially useful in making mass inoculations such as the seeding of large tubes and plates for the production of spores to be used in various types of experimental work

Mounting Fluid

In the microscopic examination of the Aspergilli it is often satisfactory to mount conidia heads or other structures in water It is more generally

satisfactory, however, to use some mounting fluid of a composition designed neither to swell nor plasmolyze the tissues to be observed Such a mounting fluid was developed by Amann as early as 1896 and has been used by mycologists, quite generally, for many years Its composition is as follows

Carbolic Acid Crystals (c p)	20 0 grams
Lactic Acid (sp gr 1 2)	20 0 grams
Glycerine (sp gr 1 25)	40 0 grams
Distilled water	20 0 cc

The carbolic acid crystals are first liquefied by heating in a water bath

The mounting fluid is normally used without the addition of any dye since in the diagnosis of the Aspergilli the natural colors of conidiophores conidia, etc , are very important If it is considered desirable to stain the tissues under observation, it is possible to incorporate into the lactophenol solution some coloring substance such as cotton blue, eosin, or some other aniline dye In making mounts of the Aspergilli it is profitable to wet the material first with 70 percent alcohol to drive off air bubbles and then quickly add a small drop of the lactophenol prior to the placement of the cover glass

Incubators

Almost all of the Aspergilli grow well and sporulate abundantly at laboratory temperature There are, however, certain exceptions to this general rule (see p 45), and for this reason it is desirable to have available an incubator which can be regulated at temperatures below that of the laboratory and others covering different ranges up to 37° C or even 50 C The type of incubator is not critical and almost any type of cupboard or room will prove satisfactory if the temperature can be controlled to within 1 C ± and if the air is neither excessively dry nor humid to the point where cotton plugs become moist upon continued exposure

Microscopes

In the study and identification of Aspergilli it is essential to have at one's disposal a good-quality compound microscope If possible this should be provided with apochromatic lenses In our experience we have found a 3 mm objective used in conjunction with either 10× or 15× oculars to give us magnifications and a degree of definition which is most satisfactory for the examination of conidial structures While it is not absolutely necessary it is desirable to have in addition a low power wide-field dissecting microscope covering magnifications from 10 to 40 diameters In the absence of such a microscope a high quality pocket magnifier provides a good substitute

Photographic Equipment

A limited amount of photographic equipment is a very valuable aid in the study of this or any other group of molds A camera should be available which can be used in conjunction with a compound microscope and to which low power lenses can be attached directly In addition to a good quality lens producing pictures above and below natural size a series of Tessar lenses which will provide magnifications of from 5 to 30 diameters is extremely useful With the aid of these details of colony structure can often be recorded which cannot be pictured with the lenses of the compound microscope and which are very difficult to describe adequately in words With these low power lenses it is possible to photograph types of fruiting heads the relative abundance of conidial structures to vegetative mycelium, and the relationship between each of the above and sclerotia or perithecia when such structures are present

Chapter V

PRESERVATION OF CULTURES

Any mold that is valuable because it has been used in fundamental research work, because it has been found useful in some industrial process, or because it is a significant agent in some destructive or pathogenic situation should be preserved to insure its identity for subsequent use or reference Identification by description will take the careful worker to the group species, and often to the variety as based upon morphology or some conspicuous character, but the reidentification from description of the exact organism used in a biochemical investigation or discovered in some ecological situation is generally impossible Culture collections have therefore, developed The Centraalbureau voor Schimmelcultures (cited by them as C B S) at Baarn, Holland the National Type Culture Collection in London, and the American Type Culture Collection at Washington are well known sources of such material More recently established but containing a greater number of industrially important molds is the culture collection of the Northern Regional Research Laboratory (commonly cited as N R R L) at Peoria Illinois The Aspergilli are especially well represented and contained in the collection are almost all of the species considered in this manual together with records which check the identifying numbers of this collection against the records of the source collections* which were brought into it

In undertaking a comparative study of the Aspergilli, or any other group of molds it is essential to maintain in a viable state a large number of isolates representing diverse species and strains By this means it is often possible to interpret current isolates and accessions in terms of historic types and concepts In maintaining such a collection of molds the objective should be to preserve viability without growth or germination of spores during the storage period By so doing the user can reasonably expect to maintain his organism without variation degeneration or mutation A number of techniques can be employed to advantage the more useful of which will be considered in some detail with certain of their advantages and limitations noted

* Currently contained in this collection are the molds formerly maintained by Thom and Church and later by Thom and Raper, in the United States Department of Agriculture Washington D C many of the Mucorineae maintained by Dr A F Blakeslee for many years at the Carnegie Institution Cold Spring Harbor Long Island New York a large number of miscellaneous forms from the Harvard University Collection initiated by Prof R Thaxter and more recently maintained by Dr D H Linder and limited numbers of cultures from many other collaborators

Agar Slant Method

The method most generally employed for maintaining mold cultures and the one which has been successfully used by the writers for many years, may be termed the agar slant method This involves the periodic transfer of spores from old agar slants or plate cultures to new agar tubes The composition of the substratum is varied to suit particular requirements and groups of organisms In our work we regularly employ the Czapek s solution agar Few if any strains make their maximum growth on this medium but it has been our experience that they maintain exceptionally well any characteristic morphological or physiological features which may characterize them It is necessary to know the expected viability of all cultures to be maintained and to gauge the intervals of transfer accordingly With the Aspergilli transfer every 8 to 9 months is sufficiently frequent for all species with the possible exceptions of *A citrisporus* and *A itaconicus* and a period of one year is not too long for most forms In practice it is advisable to handle separately the few very short lived species and to set the regular period of transfer well within the known viability period of the remaining forms Transfer of the general collection at least once each year insures a complete survey within the yearly period of all strains maintained New tubes are inoculated at least in duplicate while triplicate preparations afford a desirable margin of safety The new cultures are incubated for 2 to 3 weeks or until a good crop of spores has developed Incubation at room temperature is suitable for most of the Aspergilli although a few forms such as the large-spored members of the *A glaucus* group sporulate more abundantly at 20 C The correctness of the cultures is then checked with a wide-field binocular or a 10X pocket magnifier the plugs are poisoned to preclude any possibility of subsequent contamination and selected tubes are placed in storage Cultures can be stored for reasonable periods at room temperature certain species will remain viable for many years at 24 to 26 C The viability of most species is materially lengthened and the possibility of progressive variation reduced by storage at 2 to 4° C (i e above any danger of actual freezing but sufficiently low to prohibit further growth and possible dissociation) Tubes of any desired size may be employed We have found lipless tubes 15 by 125 mm to be quite satisfactory since they provide adequate culture surface and at the same time require much less storage space than the larger tubes commonly in use Each culture should be maintained at least in duplicate with the different tubes of each pair stored in separate refrigerators With the accidents and failures of refrigeration the possible escape of toxic gases or the possible ingress of contaminations that escape the usual inspection the maintenance of not less than two complete series of strains is a necessary precaution If natural conditions such as temperature and relative hu

midity are favorable as at Baarn Holland, refrigeration may be dispensed with, but watchfulness against invasion by mites becomes more important Additional slants should be prepared for cultures which are frequently used

Agar slant cultures are convenient for use, easily examined and compared, and easily replaced (fig 13 A) They are, however easily contaminated when handled carelessly Uneven drying subjects the culture to

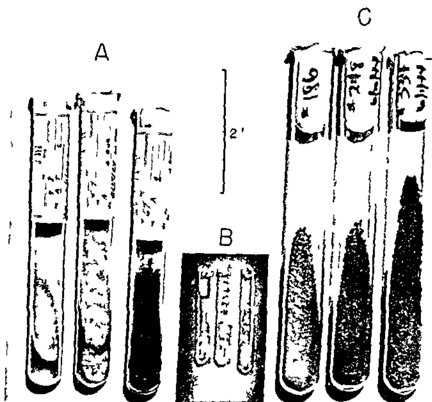


FIG 13 Methods of maintaining stock cultures as discussed in the text *A* Cultures growing on agar slants *B* Cultures preserved in lyophile form note the compact chalky pellets formed by the dried serum in which the spores are suspended *C* Cultures preserved in dry soil

extremes of contrast in concentration of media and metabolic end products between the thin edge of the slant and the heavier mass in the bottom of the tube Variations (apparent or real) often appear in the stored cultures and these may be propagated in subsequent transfers As a safeguard stock cultures should be grown in petri dishes after several transfers in tubes thus making possible more complete examination to maintain purity and typical morphology

Preservation in Lyophile Form

Studies now in progress at the Northern Regional Research Laboratory indicate that many if not all of the Aspergilli can be successfully maintained in a dried state for extended periods Viability tests for a number of species including *A terreus* *A niger*, *A oryzae* *A flavus* and *A itaconicus* have been made at 3½ years while a much greater and wider variety of forms has been tested at 20 to 24 months Positive results have been obtained with all cultures tested although comparatively few colonies devel oped from certain strains of *A niger* *A flavus* and the large-spored members of the *A glaucus* group Observations are being continued and in time information will be obtained as to the feasibility of employing this as the principal means of maintaining a collection of molds It is known that many bacteria especially staphylococci streptococci and pneumococci can be successfully preserved for periods up to 16 to 18 years (Elser, Thomas and Steffen 1935 Swift 1937) Wickerham and Andreasen (1942) have presented evidence covering a period of one year which suggests the practicability of applying the method to the yeasts Such information as we have to date regarding the molds seems to indicate that the method may prove of great significance in two ways first as a means of prolonging viability and second as a means of preserving in viable form spores of a particular generation or other selected origin which can be used in comparative tests over a period of many months or even years

The drying technique employed at the Northern Regional Research Laboratory is essentially like that described by Wickerham and Andreasen (1942) and may be briefly summarized as follows

Employing aseptic techniques throughout the spores from selected cultures are suspended in sterile beef or horse serum The resulting suspension is then dispensed into small cotton stoppered Pyrex glass tubes 6 mm by 100 mm that have been properly labeled with glass-marking ink Approximately 0 05 to 0 1 cc of the spore suspension is added to each tube by means of a long thin necked pipette Most of the cotton plug is burned away and the remaining portion pushed down into the tube to prevent possible contamination during the drying process The tubes are inserted in rubber sleeves on the manifold as shown in figures 14 A and B and lowered into a freezing bath of carbon dioxide ice and methyl cellosolve at a temperature of approximately −40 C The suspension is frozen almost instantaneously The manifold is connected to a vacuum pump and evacuation and desiccation initiated Water is removed from the system by the insertion of a water trap immersed in a CO_2-methyl cellosolve filled Dewar flask as shown in figure 14A or in a column of drierite (anhydrous $CaSO_4$) as shown in figure 14B After a few minutes the temperature of the bath

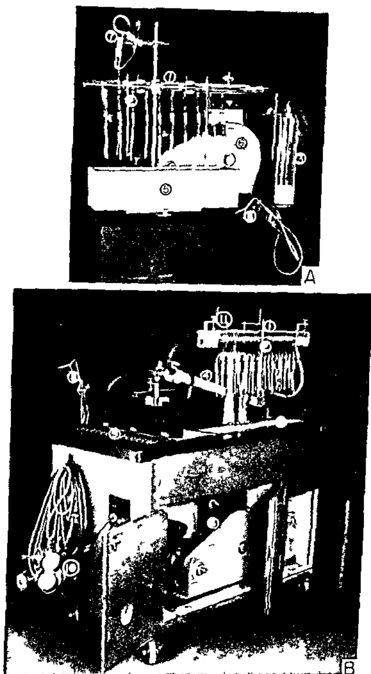


Fig 14 Apparatus employed at the Northern Regional Research Laboratory for preserving microorganisms in lyophile form *A* Table model of thirty tube capacity utilizing a trap immersed in a Dewar flask filled with CO_2 ice and methyl cellosolve to collect the water vapor removed from the drying preparations *B* Larger self

surrounding the tubes is raised to approximately −5 C[1] by su[p]
additional methyl cellosolve that has been precooled to approximatel[y]
The bath is maintained at this level continuously until the prepa[rations]
appear thoroughly dry In drying the serum contracts slightly to [a]
well-defined chalky pellet When the pellets are apparently dry, th[e tubes]
are raised above the bath and evacuation is continued for one-half to [three-]
quarters of an hour at room temperature to insure as complete rem[oval of]
water as possible, after which time they are sealed off with a gas-[oxygen]
torch (fig 14 A) On the following day each tube is tested for the pr[esence]
of a good vacuum by means of a high frequency spark coil tester, an[d only]
those tubes which show such a vacuum are retained Tubes not ma[intain-]
ing a satisfactory vacuum are very rarely encountered in actual pr[actice]
Quadruplicate tubes are regularly made for each stock culture, a[nd the]
finished preparations are stored in a refrigerator

In recultivating the molds, the tubes are marked with a file scratc[h, sur-]
face-sterilized and the tube broken inside a wrapping of sterile [...]
The content which is in the form of a well formed pellet (fig 13 B), [is]
dissolved in 1 to 2 cc of sterile broth or water This is streaked o[n]
plates and colonies are allowed to develop New isolations can be [made]
within a period of a few days It is possible of course to go directl[y from]
the lyophile tubes into flasks or other cultures used in actual experi[ments]
but generally speaking much larger quantities of material would [...]
be processed

The feasibility of preserving molds in lyophile form over long [periods]
has by no means been proved but results to date are very encou[raging]
Should it be found that spores of molds like bacterial cells can b[e kept]
viable by this method for many years it will prove ideal as a means [...]

[1] Wickerham and Andreasen in 1942 governed the temperature at which t[he sus-]
pension was dried by adjusting the level of the tubes above a bath which w[as held]
at a very low temperature Subsequent to this Wickerham developed the pr[ocedure]
outlined above

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contained and portable unit of sixty tube capacity (designed by Dr L J Wick[erham]
which utilizes a column of anhydrous calcium sulphate (drierite) to collec[t water]
vapor removed from drying preparations A_1 and B_1 Glass manifolds [...]
Thermometers, A_3 Dewar flask containing water vapor trap immersed [in dry]
ice and methyl cellosolve, B_3 Column of drierite A_4 and B_4 Freezing ba[th con-]
taining CO_2 ice and methyl cellosolve in which preparations are immer[sed for]
quick freezing and subsequent temperature control A_5 and B_5 Vacuum pu[mp ...]
Vacuum guage (mounted at opposite end of apparatus shown in figure B) A_6 [...]
Gas-oxygen torch for sealing off dried preparations B_6 Terminals on which [...]
tubes are mounted to be tested for presence of good vacuum by means of a h[igh fre-]
quency spark coil tester (not shown), B_6 Oxygen tank B Screw lift for [raising]
and lowering manifold and attached tubes (In figure 4 manifold is raised and [...]
manually and locked into position by means of a wing bolt)

serving large culture collections It possesses certain marked advantages

(1) There is no possibility of contaminants entering the sealed preparations

(2) The investigator recultivating the molds starts with the actual spores contained in the original suspension

(3) The space required for storage of a large number of lyophile preparations is much less than for any other type of culture (fig 13 B)

Preservation in Soil

Soil has been successfully employed as a means of preserving vigorous stock cultures over long periods As early as 1918 Barthel (Cent Bakt *II 48 340–49 1918)* reported the successful maintenance of yeasts and bacteria in this medium and modifications of this technique are now employed in many laboratories for preserving bacteria Greene and Fred in 1934 compared cultures of various molds preserved for two years in soil with the same strains continuously maintained on malt extract and malt extract-potato glucose agars and on bread In their experience, soil preparations were most satisfactory and Professor Elizabeth McCoy (personal communication) has recently reported cultures of *A sydowi* preserved in this manner to be viable after nine years Since the publication of Greene and Fred s work, the soil method has been rather generally used by the Wisconsin group as a means of preserving valuable stock strains of molds Furthermore it is known to have been successfully employed during the past two years by a number of laboratories to maintain cultures of penicillin producing molds in a high and uniform state of productivity The soil substrate used by Greene and Fred was prepared as follows

To air dried orchard loam soil (Miami silt loam) sufficient water is added to bring it to a moisture content of about 20 percent The soil is then transferred in convenient amounts (about 5 grams on a dry basis) to ordinary half inch (1 27 cm) culture tubes The tubes are plugged with cotton and given four 3 hour sterilizations at 15 pounds per square inch (1 kg per sq cm) pressure on alternate days and tested for sterility by addition of yeast water glucose broth to tubes selected at random The tubes are then inoculated with 1 cc of a heavy spore or mycelium suspension of the desired mold and kept at room temperature That there is appreciable growth and sporulation on the soil can usually be ascertained without difficulty by direct microscopic observation *While the addition of nutrient to the soil may* bring about somewhat greater growth it does not seem to enhance the keeping qualities of the cultures

It has been found possible to preserve on soil mold stocks used for large scale growth—namely *Aspergillus fischeri A sydowi* and *Penicillium chrysogenum*—for over 2 years without loss of their essential and desirable characters Moreover the gross colony characters have remained much more constant than did those of the corresponding cultures maintained in the usual way on agar slants The soil cultures

can be recovered as required simply by streaking some of the soil particles on fresh agar slants

It is not in all cases advisable to depend on soil alone for the preservation of valuable stocks but reserve stocks may without difficulty be prepared on soil and the writers believe that in many instances soil will be found to be an excellent medium for maintenance with a minimum of change over long periods of time

During the past two years the soil method has been used at the Northern Regional Research Laboratory with but minor modifications of the technique cited above (fig 13 C) Its principal advantages lie in the fact that (1) the viability of strains is apparently lengthened and (2) from a single stock tube opened with proper care repeated cultures can be started simply by removing some of the soil particles to suitable substrata

Vegetable Substrata

The oriental fermentation industries maintained their inoculating material as selected rice or soybeans upon which the mold had been grown under favorable conditions to produce maximum quantities of spores This nutrient dried and packaged was stored and sold under the Japanese name Koji Samples examined after several years showed excellent viability Bainier was a pharmacist He distributed licorice root in sections 5 to 10 mm in diameter and 5 to 8 cm in length in test tubes, sterilized them and kept his cultures regularly for years upon them Tested by us the method was a very satisfactory laboratory practice American mycologists have successfully used bean stems for the purpose Apparently any organic material which provides frameworks of cellulose enmeshing sufficient nutrients to support mold growth without complete breakdown of the mass may be used

CONTAMINATION

Mixed Strains

In the routine conduct of cultural work contaminations of cultures of one species of Aspergillus by other species or species of other genera is very common The conidia of most molds are exceedingly light and are carried freely in the air Entire exclusion of such contamination is difficult In dealing with contaminations several problems arise and different procedures are possible A colony of a single Aspergillus well established usually inhibits the growth of other species developing in the immediate vicinity Even if invasion occurs the effects are commonly so distinct as to leave little doubt as to the limits of the different forms When however the contamination with spores or mycelium is carried in the inoculum and so placed as to germinate in intimate contact with the organism desired (1) the species may sector out and hence be easily recognized or (2) the colony

Mites

Mites are very common in rotting vegetables, especially in partly dried condition in dried meat products, in hard cheeses, and in organic soil masses They are thus common associates of molds as they occur in nature



FIG 15 *Penicillium* sp parasitic on *Aspergillus niger* × 165 (Photograph by Edward Yuill)

hence the worker who handles moldy substrata in isolating his organisms must constantly watch for them In size mites are commonly just about at the limit of visibility by the unaided eye One accustomed to them will detect them readily but until seen and the appearance of their depredations

understood, they can pass unnoticed for considerable periods by persons who are otherwise good culture workers Mites will crawl from petri dish to petri dish leaving behind them a trail of bacterial and mold contaminations as well as streams of eggs which develop rapidly into more mites that actually destroy the colonies Since mites have preferences as to food some species are invaded and others avoided To reach an attractive food supply a mite will frequently go through a cotton plug as ordinarily made and occasionally seems to get through even a paraffined plug As a factor in the mixing of strains of molds in a laboratory collection mites must not be ignored

Similar mixing and contamination occurs frequently whenever a laboratory becomes infested with ants roaches or other insects

Poisoning Cotton Plugs

Mites Entire elimination of mites by sanitary measures is possible but often not attained As a precaution in the preservation of stock cultures some scheme of poisoning should be used One of these formulas consists of dipping the tips of the cotton plugs in a solution of the following composition

95% alcohol	95 cc
Bichloride of mercury	0 5 gm
Glycerine	5 cc
Color with any aniline dye	

Care must be taken that the solution does not come in contact with the colony The cultures must be allowed to develop into typical colonies before poisoning An antiseptic formula for the purpose needs alcohol to insure penetration of the plug a poison to destroy the mites glycerine to prevent the crystallization of the poison as the alcohol evaporates and the dye to insure the destruction of the cotton plugs when removed from the tubes In our own experience we have consistently made it a practice to wipe off the outside of all culture tubes with the above or some other sterilizing solution and to poison all plugs before cultures are replaced in or added to the collection of stock cultures

Molds Under humid laboratory conditions cotton plugs, especially if made from the absorbent type of cotton absorb moisture Careless handling in preparation and care of such plugs often adds enough nutrients to support growth Steam sterilization tends to distribute nutrients Dirt bacteria and molds fall from the air upon the exposed portion of the plug Handling detaches spores from the colony within so that both ends of the plug are commonly well seeded Spore germination therefore may begin at either or both ends Outside molds may grow through and drop into the

culture, or the culture itself may grow out through the plug and contaminate other cultures or experiments Surface sterilization of the outside of the tubes and poisoning the plugs takes care of molds, as well as mites

Spraying Oily sprays, as selected fractions from petroleum, available from commercial sources, even kerosene distributed with a gun that produces a mist penetrating and filling all cracks crevices, open spaces among apparatus or furniture and clouding the whole atmosphere of the laboratory, have been found effective in carrying down mold spores and bacteria from the air and ridding the laboratory of mites, insects, and vermin

PRESERVATION OF DRIED SPECIMENS

Mold cultures lose many of their characteristic and diagnostic features upon being dried Nevertheless dried herbarium specimens serve a useful purpose in preserving type material which might otherwise be lost Details of morphology are often difficult to establish from such material, but group characteristics are preserved and over all colony appearances can be recognized after many years The retention of culture tubes or petri dishes containing such dried specimens constitutes a reasonably satisfactory means of preservation, and the material contained therein approximates as nearly as is possible the cultural picture of the growing colony Glass tubes and dishes however are cumbersome and easily broken hence may prove unsatisfactory if frequent handling is necessary For many years we have employed an alternate technique with generally satisfactory results Representative portions of colonies grown in petri dishes are cut with a large cork borer lifted out with a spatula and dropped into paper pill boxes where they *are allowed to dry These can then be stored in* larger boxes or attached to herbarium sheets for filing The boxes should be provided with tight fitting lids, and for greatest convenience should measure approximately 1½ inch in diameter Aspergilli stored in this way prove useful in many comparative studies They cannot however under any condition take the place of carefully handled living cultures

Chapter VI

VARIATION

The Aspergilli are a variable and mutable group of fungi They are characterized by great diversity and variability as they are isolated from nature and an increasing amount of evidence shows that they can be made to vary or mutate in the laboratory by subjecting them to a number of different imposed stimuli Frequently the same types of mutants or variants ultimately result under both natural and artificial conditions Nevertheless it is believed desirable to consider somewhat separately variations and mutations resulting from natural causes and those resulting from imposed stimuli

Definition of Terms

Before entering upon a discussion of variation either natural or induced it is important to define certain terminology which is to be employed It is recognized that our definitions will not agree in all cases with those of earlier workers nor do we expect that all subsequent investigators will accept those which we propose If the meaning in the present discussion is clear our purpose will have been served

(1) The term *mutant* or mutation is used to designate a strain whose source is actually known and can be verified Furthermore it is limited to those substrains which originated as sharp breaks from parent cultures (usually interpreted as gene mutations) and in successive culture generations retain their distinguishing characteristics unaltered This may or may not have a taxonomic connotation Upon occasion it is used in essentially the same sense as variety To illustrate *A nidulans* mut *albus* *A fumigatus* mut *helvola* *A niger* mut *cinnamomeus* etc are used as Latin names to designate forms which differ from the parent species in certain striking details In other instances it is used to identify a type of change rather than to designate a particular and isolated strain resulting from such change It is considered correct to refer to artificially produced albino yellow and buff-colored strains of *A terreus* as mutations (see p 75) since they are constant in character and are known to have originated from a cinnamon-colored parent culture wholly representative of the species and we believe it represents good judgment to refrain from assigning Latin designations to each of them The term mutation then refers to altered strains of constant character and known lineage whether or not they are given Latin designations

(2) The term *variant* or variation is used loosely and reference to it in this manual is not, in all cases, entirely consistent. In general however it is applied to subcultures, or strains, arising through gradual change from well-defined strains of identifiable species. The characters of a variant, then, are not generally stable but subject to continued change and further variation. As used by us the term has no taxonomic implication and can be considered essentially synonymous with the term 'saltant' which is so commonly employed in reports on variation in the *Fungi Imperfecti*. Variants frequently appear as colony sectors, overgrowths, or other localized areas of changed appearance or texture. When isolated in pure culture they may or may not retain their distinguishing characteristics.

NATURAL VARIATION

Cosmopolitan species and groups of Aspergilli show adaptability to wide ranges of environmental conditions. As these molds are isolated from nature variation among the members of any species series *or group is* regularly encountered. Such variations commonly differ in degree rather than in basic characters and one can distinguish a series of intergrading or bridging forms. Even striking isolates are often unmistakably allied with some well defined species or group in this manner. Such different but intergrading forms arising in nature can be considered as natural variants. Natural variants of a similar kind can frequently be obtained in laboratory culture by selective isolation and cultivation from sectors or other areas of atypical growth, or by single spore isolations. Distinction must be drawn between differences in appearance, morphology, and habit of growth resulting from inherent differences between strains, and alteration in colony character in response to changes in the composition of the culture medium or other environmental factors. Rigid comparative culture is often necessary to distinguish between the two. For the present discussion we are concerned with differences that are more fundamental than direct temporary responses to artificial stimuli (ecads), but the latter unless carefully evaluated may appear no less real. Rightly or wrongly Blochwitz (1930, p 247) comments that *A flavus*[1] in specimens collected in the Botanical Garden at Buitenzorg was called *A penicillopsis* (Henn.) Rac. in the Botanical Garden at Singapore *S vitellina* Ridley, in India *S corolligena* Massee, and in Columbia *A delacroixii* Saccardo.

Occasionally isolations are made from laboratory cultures which represent sharp breaks from the parent strain and since they are constant in subsequent culture they may be considered as true mutations. Representatives

[1] The name of the original describer is only used for specimens or strains in culture which were definitely attributed to the describer. *A flavus*, *A niger*, *A terreus*, etc are series concepts as used here.

of such natural mutations which originated in the absence of any artificially imposed stimuli are Yuill's *A fumigatus* var *helvola* (1939), *A nidulans* var *albus* (1939) and *Cladosarum olivaceum* (1938)

Intra strain Variation

A certain amount of variation can be expected to occur in any given strain of Aspergillus In certain species and strains this is very limited and cultures can be re-cultivated repeatedly upon a variety of media and at different temperatures and H ion concentrations without evidences of visible change other than those resulting from the immediate effects of the altered environment Many strains of *Aspergillus niger* are characterized by such comparative stability Other species and strains are subject to continual variation with differences in character and rate of growth appearing rather abruptly as sectors or overgrowths or gradually developing as a progressive alteration in the general aspect of the whole culture By successive and selective subculturing strains of *A alliaceus* and *A ochraceus* showing a marked difference in sclerotium production can be obtained In like manner strains of *A itaconicus* Kinoshita can be secured which are almost completely sterile upon all media tested The same is true of *A granulosus* Raper and Thom *A flavipes* etc

Working with a strain of the ascosporic species *A fischeri* Greene (1933) isolated 448 single spore cultures and among these found variant progeny of two main types (1) cultures producing very large scattered perithecia as opposed to the typical picture of many small perithecia and (2) cultures producing conidial structures in profusion but forming few perithecia and these tardily The second type was fairly stable whether derived from single ascospores or conidia The first type was variable in some cases reproducing the characters of the variant parent in others reverting to the character of the original stock culture

Hansen (1938) and Hansen and Smith (1932) have studied many of the Fungi Imperfecti rather exhaustively and report that single strains of these fungi are basically composed of a mycelial (M) type and a conidial (C) type By proper techniques the two forms can be separated and recombined at will While it has not been explored as yet the possibility exists that the same condition may prevail to a limited degree in the Aspergilli and that this may account for a certain amount of the intra-strain variation encountered

Back of variability in molds many lines of discussion have been developed Buller (1933) has frequently called attention to the unmeasured possibilities of nuclear and cytoplasmic disturbance from the commonly observed phenomenon of anastomosis Vegetative hyphae belonging to the same mycelium (mycelium derived from a single spore) or different mycelia

throw out branches which fuse without showing any other sign which might suggest a sexual process either before or after fusion Anastomosis is usually observable, if at all, in the rapidly growing area where many spores placed as an inoculum are developing into one colony By transferring large numbers of spores from an old culture to a new one, most of the Aspergilli studied by us have shown fairly consistent repetition of colony characters and conidial morphology and have been maintained for a long time with little or no observable change Other organisms handled in the same manner have not been successfully maintained with the morphology originally studied Differences in behavior can be attributed to variability between strains

Intra species Variation

Variation within the species is very prevalent and is well marked in many cases When a large number of isolates of any particular species or series is collected, one can regularly expect to find among them wide variations in color, amount of sporulation, and in their general habit of growth Usually, however, such variation is graduated and strains representing various intermediate steps between the extremes are to be expected While it is by no means unique, we may use *A terreus* as an example since a very large number of strains belonging to this species have been isolated and observed in plate and tube cultures during the past two years (fig 16) Colonies of the type strain and of the great majority of isolations made from nature, are plane, cinnamon in color, very heavy sporing with conidial heads arising directly from the substratum in an even and close stand An occasional isolate is much brighter in color approximating xanthine orange (Ridgway Pl III) but in all other respects it is fairly typical It is believed to represent a form such as that described by Blochwitz (1934) as *A boedijni* and in the present manual we have designated it as *A terreus* var *boedijni* In each of the above cases, the production of abundant fruiting structures follows closely the advancing margin of the growing colony and there is little or no continued growth of mycelium except in the marginal area Certain other strains are quite floccose conidial heads are typical in form and color but are greatly reduced in number and are borne upon aerial hyphae Shih (1936) was probably working with such a culture when he described the variety *A terreus* var *floccosus* We feel that the forms are sufficiently distinct to warrant maintaining his variety Still other strains possess abundant but fairly close- rather than loose-textured mycelia and bear abundant but very pale buff-colored conidial heads The vegetative mycelium in this form is bright yellow and for this reason we have designated it as *A terreus* var *aureus* n var (see p 198) If one should examine only the type strain and these three atypical forms it is entirely probable that one

would describe them as four distinct species. Actually, however, they are not sufficiently distinct to warrant specific rank, for they represent only extremes of variations along three divergent lines with numerous inter-

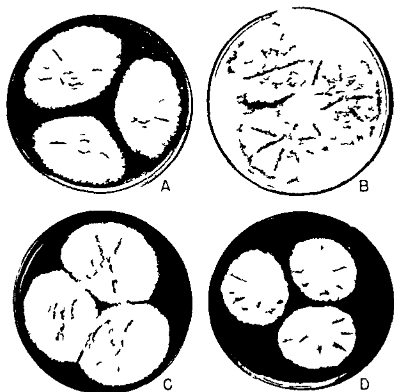


FIG. 16. Intraspecies variation in *Aspergillus terreus*. A, *A. terreus*, typical strain NRRL No. 265, characterized by heavy conidium production and colonies cinnamon in color. B, *A. terrreus* var. *boedijni*, NRRL No. 680, characterized by deeper colonies and conidial heads near xanthine yellow. C, *A. terreus* var. *floccosus*, NRRL No. 1921, characterized by loose floccose colonies and spore heads light pinkish cinnamon in color. D, *A. terreus* var. *aureus*, NRRL No. 1923, characterized by yellow floccose colonies and comparatively few cream to buff-colored conidial heads.

grading strains aligning them almost without interruption with the typical form itself.

Another series of variants in our collection shows gradation from the usual radiate head and conidiophore of *A. sydowi* to the simple mono verticillate penicilli of *Penicillium restrictum* Abbott. The tendency to form

reduced conidial apparatus is observed in all of the strains of *A sydowi* examined Over a period of many years variants have exhibited all gradations in colony appearance from typical *A sydowi* to the aspect of *P restrictum* of Abbott except for the presence of an occasional conidiophore and head of *A sydowi* Repeated cultural tests exclude contamination These variants occur in nature, they produce abundant conidia, and they undoubtedly maintain themselves successfully in the field

Aspergillus fumigatus presents a somewhat similar condition In this species, typical cultures produce heavily sporing, velvety colonies that are dark green in color and show almost no aerial mycelium Other strains commonly isolated from nature produce very floccose colonies and bear comparatively few conidial heads (fig 37) These heads however, are typical in form and in dimension Strains possessing this contrasting character are relatively stable in culture but in this species as in *A terreus*, all degrees of intergradation are found between this floccose type and strains entirely typical of the species The same story is repeated in other species

Appreciable variation can normally be expected among the isolates of any of the very abundant and cosmopolitan species Such variant strains however are the exception rather than the rule since the great majority of isolates are quite typical of the species Attention was called to this fact in our study of the *A glaucus* group (1941)

When grown in comparative culture strains successively isolated as representing a particular species usually show enough difference to give each strain a kind of individual ty Exact identity point by point is not expected Such strain variation may be incidental and unimportant or it may be correlated with activities which make one strain a valuable agent in an industrial process and the other worthless

Intra group Variation

Inside the different groups of Aspergilli one normally finds somewhat similar but wider variations than those seen among the strains constituting any particular species To what degree species in nature have developed by mutations and by progressive variation can only be guessed We do know however that the species within a group like the strains or varieties within the species are regularly bridged by intermediate forms which render it difficult to establish sharp and immutable lines of separation One can almost cite it as a rule that the definiteness with which one regards a species is inversely proportional to the number of strains of that species which have been examined Still species are necessary as guide-posts—as fixed points around which closely related organisms showing a certain but limited amount of variation can be grouped

The *Aspergillus flavus oryzae* group can be taken as illustrative of the type of variation to be expected within a group of the Aspergilli Thom s

culture No 113 (NRRL No 447) of *A oryzae*, received from Baarn and believed to stem from Cohn's original strain is a very floccose loose-textured culture bearing comparatively few small light yellow to tan heads Conidiophores are long ranging up to 2 to 5 mm and are very thin walled There is only a trace of green even in individual heads and in general aspect the culture normally shows no green color *Aspergillus flavus* in its typical form is not floccose and is very heavy sporing Conidiophores arise directly from the substratum in a close stand, are usually 1 mm or less in length and are comparatively heavy walled Colonies are regularly in yellow green shades and range from light to comparatively dark green (see species description) *A parasiticus* Speare goes even farther Colonies are very dark green in color Conidiophores usually range from 200 to 400µ in length and sterigmata are typically in a single series whereas they may be in a single or double series in *A oryzae* and *A flavus* In the opposite direction but markedly different from *A oryzae* is *A effusus* Tiraboschi Typically this is very floccose and comparatively light sporing with heads borne upon short conidiophores which arise from the loose aerial mycelium rather than from submerged mycelium in the substratum

The cultural pictures of these species are fairly characteristic Yet it is practically impossible to take a large collection of 100 or more strains and separate them into these species with any degree of confidence or satisfaction The difference between *A oryzae* and *A flavus* is bridged completely by a series of intermediate forms showing all degrees of variation between the two strains selected as typical Nomenclature in this group is then further complicated by the fact that among the great collections of these forms obtained from the Orient and designated *A oryzae* the majority of forms are somewhat intermediate between *A oryzae* and *A flavus* as depicted above A similar series of intermediate forms bridges completely the gap between *A flavus* and *A parasiticus* There is no sharp line of demarcation between any of these species still they are not one and the same and to attempt to lump these diverse forms together into one species as Neill (1939) has done for the *A glaucus* group intensifies rather than reduces the difficulties encountered

Intra-group variation is also particularly marked in the *A niger* group During an extended period of study and observation of molds in culture Biourge who was a discriminating collector accumulated 63 strains of black Aspergilli which suggested sufficient individuality to be deemed worthy of further study These were turned over to Mosseray when he entered Biourge's laboratory He assumed that he had before him all of the black Aspergilli possible to collect and knowing that Biourge had selected each of them because it seemed to have some special character he undertook a taxonomic study to define those characters and to organize them into a systematic presentation (1934a) His paper lists 35 species of

which 25 were either described as new species or new combinations His findings in *A niger* are fairly illustrative of the same type of study in other groups (compare Thom and Currie [1916] for *A niger* Thom and Church [1921] for *A flavus*

Mosseray based his primary separations upon conidial sizes, shapes and markings, while secondary and tertiary separations were based upon conidiophore lengths and the characters of colonies in tube cultures on Biourge's "Raulin neutre gelose —a variation of the classic Raulin solution Biourge and Simonart demonstrated the entire series to one of us (C T) showing how wrinkling and granulation of mycelium, intensity and changes of secreted color, shades of color in the conidial area lengths and proportions of conidiophores and heads, and their distribution over the mycelium gave to each strain an individuality which had been repeated in successive cultures over a considerable time We raised just one question, 'What would you do with the next thousand? '

The large majority of all isolations of black Aspergilli conform within a range of minor variation with the general van Tieghem concept,—i e black brown colonies with conidiophores and heads giving the general structure and measurement of parts found in the classical description Then, in contrast with these, there are shades of colony color from the coal black of *A carbonarius* through shades of purplish black to the brown of *A ferrugineus* Fuckel or to the lighter shades of Schiemann s mutants (pp 223–224) Some of them produce no colors in the substratum and reverse of the colony some show yellow in traces others are persistently deep orange, giving the whole a yellowish appearance Or, again the agar and mycelium may develop a red brown or mauve shade of violet

Conidiophores in the usual type of culture reach nearly enough the same length to give the effect of a field of grain But their length may be quite short and the heads seem to be borne directly on the substratum or they may be several millimeters in length with the heads borne well above the substratum and correspondingly large Between these extremes every variation can be found Conidiophores may be scattered thinly over the vegetative mycelium collected in a zone at the border or crowded in the center

The vegetative mycelium may grow as a flat felt (plane) or may be variously wrinkled sulcate or buckled In a smooth or plane colony the mycelial cells seem to stop growing early—the colony extends only at the margin In the plane colony intercalary growth (i e the formation of new cells in the filament or the lengthening of the old cells), and the production of new branching ceases Such mycelia ordinarily produce one crop of conidial heads beginning at the center of the colony and progressively developing toward the margin until the medium is exhausted or some inhibiting factor paralyzes growth Marginal growth in such a colony

often shows longer and fewer conidiophores and larger heads than in the central area

Within the group with the usual structures still recognizable many variants with contrasting features appear Heads with very long primary sterigmata which are sometimes septate appear in *A carbonarius* (Bainier) Thom *A pulchella* Speggazini or *A tubingensis* Mosseray The primary sterigmata may grow out into sterile filaments as in Mosseray s figure for *A ficuum* (Reich) Henn much more commonly some primary sterigmata grow out as tiny conidiophores and produce little heads often consisting only of a cluster of simple sterigmata and conidial chains Thus sterig matic changes may run from the simple sterigmata of *A japonicus* Saito *A luchuensis* Inui or *A malvaceus* Mosseray where only some are double to other species showing the widest range in length and arrangement

Another group of variants show marked suppression of the ordinary structures expected Strains in which conidiophore formation has been reduced or almost suppressed have been studied in continuous culture Such colonies showed an occasional long conidiophore and large head con forming to the *A niger* pattern produced at the end of the colony growth period Meanwhile the mycelium was fully covered with irregularly branching hyphal elements bearing single sterigmata variously placed groups of sterigmata or penicilloid clusters of sterigmata each bearing a short chain of conidia showing the characteristic markings of the group Transfers from the simplest form developed the complex or *A niger* ele- ments Transfers from the large heads brought a recurrence of the re- duced type of fruiting No method of selection tried brought back the typical *A niger* a pect and cultures of this type appear to represent de- generate forms

It would appear then that a general type or morphological picture when found dominant in large numbers of natural isolates can be regarded as typical for a species of Aspergillus It is recognized that marked diver gences from such types occur under the unrecorded stimuli of nature Some of these forms succeed in establishing themselves as permanent elements of the microflora and thus become successful as species or va rieties Others do not digress quite so markedly and thus constitute intermediate or bridging forms At the same time marked changes can be induced by the application of artificial stimuli Where the origin of such altered strains is known they are commonly regarded as mutants Were they isolated directly from nature and their previous history not known it is probable that they would be considered as separate varieties or even species

Natural Mutation

While most of the mutants which we recognize as such have originated in the laboratory as the result of certain artificially imposed stimuli (or

drastically altered conditions of growth), and hence can properly be termed induced mutations a number of well authenticated cases of natural mutation are known In 1939 Edward Yuill described as *A fumigatus* var *heliola* a buff colored mutant of this species isolated by him in 1937 (fig

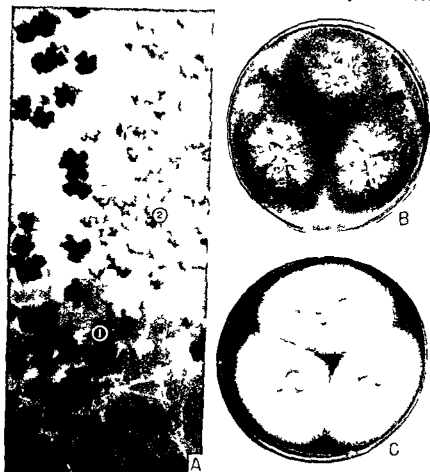
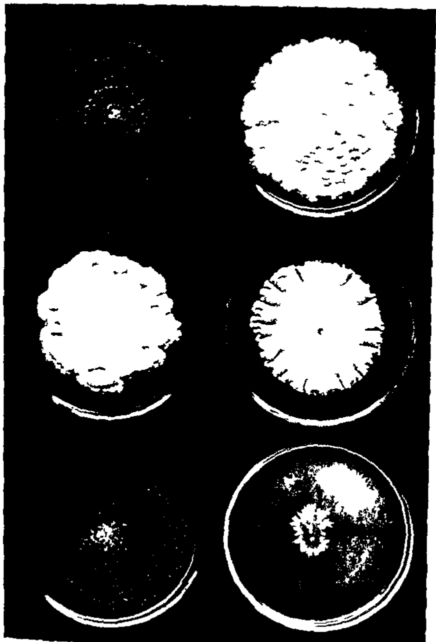


FIG 17 Natural mutations *A* Portion of a colony of *Aspergillus niger* in which a tan spored mutation appeared as a V sector A_1 typical black head of parent strain, A_2 tan head of naturally occurring mutation × 25 approximately *B* and *C* Typical strain of *Aspergillus fumigatus* and a naturally occurring mutation discovered and described by Edward Yuill as *Aspergillus fumigatus* mut *heliola* The mutations in both species have proved completely stable in continued culture

17 C) In the same report a white-spored mutant of *A nidulans* isolated in 1937 was described as *A nidulans* mut *alba* In both cases the mutants developed as natural phenomena without the application of any artificial stimuli—in the former case as a single head in the later case as a group of heads and in both cases from wholly typical strains (fig 17)



P [illegible] r II

M[illegible]tat[illegible] [illegible] *A[illegible]pe[illegible]gill[illegible] terr[illegible]* prod[illegible]ce[illegible] by [illegible] [illegible] [illegible] C[illegible] [illegible] row[illegible] [illegible] C[illegible] [illegible] sol[illegible]tio[illegible] ag[illegible] [illegible] te[illegible] perat[illegible] [illegible] eek[illegible] A [illegible] the left [illegible] NRRL [illegible] 265 [illegible] *B* [illegible] All [illegible] prod[illegible] [illegible] has [illegible] left[illegible] floccose [illegible] *D* [illegible] right[illegible] [illegible] tant prod[illegible] [illegible] left[illegible] [illegible] *F* [illegible] [illegible] y few [illegible] see text p[illegible] 5[illegible] [illegible] N[illegible]rth[illegible] R[illegible] search Laboratory[illegible] R[illegible] prod[illegible]ced th[illegible]o[illegible]gh co-ope[illegible] [illegible] & Co[illegible]

In studying a group of cultures three years ago, the authors noted a few tan heads in the form of a V-sector in an otherwise typical black colony of *A niger* (fig 17 A) Isolations from a tan head reproduced the mutant head characters and repeated transfers of this strain have proved consistently stable over a period of three years The mutant strain cannot be distinguished from *A niger* mut *cinnamomeus* (*A cinnamomeus* of Schiemann)

In dealing with natural as well as induced mutations we have endeavored to limit the use of the term mutant to forms whose origin was definitely known Blochwitz (1934, 1935) was not so precise Under the name *A glaucus* mut *alba* he refers to a white-spored member of the *A glaucus* group He believed *Aspergillus giganteus* Wehmer to represent essentially a long-stalked *A clavatus* hence designated it *A clavatus* mut *giganteus* This treatment may be justifiable We believe however that until their origin from other and well marked species can be proved it is wise to continue to recognize as species these very distinct forms that are isolated from and are able to maintain themselves in nature

Cladosarum The most striking variant or mutant ever described in the Aspergilli is *Cladosarum olivaceum* of Yuill and Yuill (1938) This appeared in a culture of *A niger* growing on bread at 28 C (Personal correspondence) Its colony conidiophores vesicles and primary sterigmata are those of *Aspergillus* The secondary sterigmata instead of producing conidia thrust out cells which are essentially the same in morphology as the secondary sterigmata themselves the same procedure is then repeated several times Occasionally however a terminal cell changes and thrusts out several equal cells in other words it resumes the function of a primary sterigma The new secondaries repeat the process of producing chains of cells each resembling the basal cell with the aspect of a sterigma not a conidium and always with the youngest cell at the tip of the chain In *Aspergillus* the sterigma which produces a chain of conidia always produces the new conidium at the base of the chain showing the next most recent farther out Differing then from Yuill s interpretation *Cladosarum* produces no conidia however readily any cell detached from the mass may grow

In *Aspergillus* the ordinary *nuclear procedure in conidium formation* involves mitosis in the sterigma actually producing the conidia After each mitosis one daughter nucleus migrates through the tube into the new spore in which it rests until that spore begins to germinate The other nucleus remains in the sterigma and repeats the process This goes on until there may be a chain of 200 conidia—the oldest at the outer end the newest directly attached to the sterigma

In the absence of cytological study one may offer the following hypothesis In Cladosarum the nuclear procedure must be reversed

The same mitosis occurs One active and one resting nucleus result, but the resting nucleus remains in the sterigma while the active nucleus moves into the newly forming cell This determines the course of development The active, multiplying nucleus is always in the newest cell formed

Previously Barnes (1928) had stimulated a strain of the *A glaucus* group (identified by us as *A amstelodami*, 1941) by heat, and reported certain mutants which were deposited with Dr Westerdijk at Baarn Among them, under the designation "Creamy" (NRRL No 143), a mold with the *morphology of Cladosarum appeared It* was obviously derived from some *A glaucus* strain and is, in so far as the writers are aware, the only other appearance of the Cladosarum structure ever discovered Barnes does not appear to have recognized its contrasting structure

Since no collector has reported this type of mutant in nature, it must either be very rare or be unable to maintain itself in a competitive environment However readily such mutants may be maintained in the laboratory, they would rarely reach the second generation in nature on account of lack of spores The name *Cladosarum* was thus applied to a defective organism (zoologically designated a "monster"), which does not become a component of any natural flora hence taxonomically the name should be untenable

INDUCED VARIATION

Striking mutations have been obtained from various species of the Aspergilli by subjecting them to artificially imposed stimuli Schiemann (1912) was among the first to draw attention to the possibilities inherent in this approach By subjecting a strain of *A niger* to various concentrations of potassium bichromate she was able to produce two striking mutations which she designated according to color *A fuscus* (= *A niger* mut *schiemanni* of this manual) and *A cinnamomeus* (= *A niger* mut *cinnamomeus* ibid), respectively Both cultures have remained stable in our hands and in various collections throughout the 32 years since their original isolation A third "mutation" designated *A niger* var *altipes* could not be distinguished from other strains of black Aspergilli isolated from nature—the parent strain was not seen Working with a member of the *A glaucus* group designated *Eurotium herbariorum* Wigg Barnes in 1928 reported the production of a series of variations by exposing spores to heat While there are reasons for questioning the correctness of some of Barnes interpretations and conclusions (see Thom and Raper 1941) there is evidence that he succeeded in producing a mutant which in its habit of growth and in the character of the fruiting structures developed bears a striking resemblance to a form subsequently isolated from *A niger* by the Yuills (1938) and described by them as *Cladosarum olivaceum* genus and species

new Galloway (1933) obtained marked variation in colonies of *Aspergillus terreus* by growing them upon media containing flour to which was added 0 003 to 0 005 per cent of salicylanilide

Thom and Steinberg (1939) and Steinberg and Thom (1940a, 1940b) in a series of experiments applied chemical stimulants to a strain of *A niger* (Thom No 4247 NRRI No 334) which had been in the collection many years without noticeable change From the cultures resulting Steinberg picked out and purified for study all variants he could observe with the naked eye and with the aid of a handlens In examining a fruiting area of a colony general changes were not common ordinarily an occasional head changed color long or short conidiophores appeared in spots gross malformation showed as areas of no fruit or too much fruit or color effects in the mycelium These were picked out and grown in successive cultures Expressed in terms of morphology the most striking feature of the tested culture was disturbance of uniformity The same types of changed aspect were reproduced many times In general they followed the same lines as have been described as present in the natural series selected by Biourge or in the collections of Mosseray at Brussels In general these changes were destructive in character and included large numbers of injury mutants or variants which reverted in subsequent transfer to the original aspect of Steinberg s culture Sodium nitrite was the most effective agent used Some isolations however represented clean cut mutations Strains of *A fumigatus* with albino heads were obtained Forms of *A niger* with light brown to cinnamon-colored spore heads essentially like those earlier obtained by Schiemann (1912) those obtained by Whelden (1940), and those subsequently obtained by Raper Coghill and Hollaender (see below) from members of the same group were likewise isolated These have remained stable in culture for the four years that they have been in our collection

Whelden (1940) succeeded in obtaining a series of mutants in *A niger* by bombarding conidia with low voltage cathode rays and subsequently isolating colonies which developed from such irradiated cells Forms possessing heads in various brown shades rather than black were isolated as well as one giant form with conidial structures appreciably larger than the parent By means of ultra violet irradiation of spores Raper Coghill, and Hollaender (in press) obtained mutants which produced tan-colored conidial heads but otherwise closely resembled the parent strain In a more exhaustive study of *A terreus* (Pl II A) the same investigators succeeded in isolating a number of striking and markedly different mutations These included albino forms with colorless conidial heads (Pl II B) forms with pale buff-colored heads yellow yellow white floccose forms with few and smaller conidial heads (Pl II C) forms producing very thin sparsely

sporing colonies forms producing restricted colonies characterized by the production of an excessive amount of orange brown exudate, and forms with leathery, close textured colonies bearing very few conidial heads (*Pl II, D*) Alterations in microscopic details commonly accompanied these changes in colony appearance (fig 19) In addition to the e morphological mutants, which were found to be stable when checked through ten successive transfers over a period of 12 months, various physiological mutants were also isolated These included forms unable to utilize nitrate nitrogen (Pl II, E) but *able to grow and sporulate normally upon media* containing ammonia nitrogen and a form unable to synthesize thiamin (Pl II F) Upon Czapek's solution agar containing sodium nitrate and sucrose, each of these is strikingly different from the parent strain upon

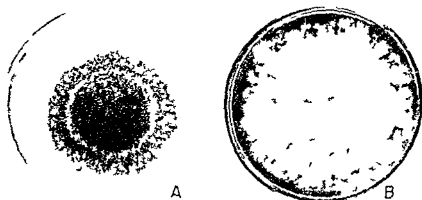


FIG 18 Induced mutation *A* and *B* *Aspergillus niger* group strain NRRL No 67 *A* parent culture growing on Czapek s solution agar 10 days room temperature *B* tan spored mutation of same produced by ultraviolet radiation

malt extract agar which contains adequate amino nitrogen and thiamin neither could be differentiated from the parent (Raper Coghill and Hollaender in press) Thus the need for comparative study and examination upon a variety of media is apparent while the importance of such studies *in reliable taxonomic work cannot be over-emphasized* In cultures of *Aspergillus terreus* resulting from irradiated pores the capacity to produce itaconic acid varied from zero in some isolations to levels somewhat above the parent culture in others The majority of such i olations produced yields somewhat lower than the parent strain many produced yields approximately equal to it while a very few produced superior yields (Lockwood Raper Moyer and Coghill in press)

Based upon our own investigations and the published reports of other

workers certain observations of a summary character can be made regarding variation in the Aspergilli

1 Aspergilli include strains and species adapted to a very wide range of environmental conditions Such conditions may influence materially the cultural and morphological characteristics of these molds

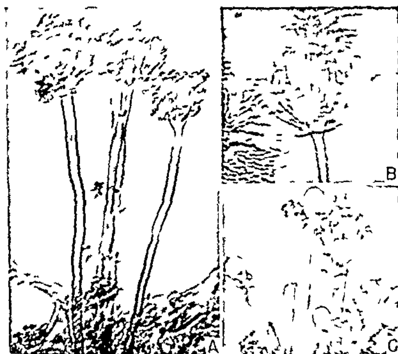


FIG 19 Photomicrographs showing details of structure in the conidial heads of the parent strain and in two selected mutations of *Aspergillus terreus* (NRRL No 265) produced by ultra violet radiation × 600 *A* Typical heads of non irradiated parent strain *B* Mutation in which conidium formation is incomplete and cells adhere in long chains in liquid mounts *C* Mutation in which many fruiting structures develop vesicles but often fail to produce sterigmata and spores

2 Under natural conditions great numbers of variants appear along with occasional sharply separable forms or mutants which are definitely of species rank The extent to which natural mutations may account for described species is a matter of conjecture

3 The Aspergilli can be made to mutate in the laboratory by subjecting them to a variety of different excitants or stimuli Induced mutations may parallel some of those found in nature and described as species

4 Particular species of the Aspergilli tend to mutate along certain definite lines, e g, the production in *A niger* of forms with tan to light brown spore heads (Schiemann 1912 Steinberg and Thom, 1940, Whelden 1940, and Raper, Coghill, and Hollaender, in press), and the production in *A fumigatus* of forms with colorless spore heads (Yuill 1939, and Steinberg and Thom, 1940a) The type of mutant produced is not governed by the type of treatment given, although the number of mutations produced is strongly influenced by this factor

5 Great variability in biochemical activity is encountered among strains isolated from nature, but these differences are rarely linked with specific morphological changes

6 The Aspergilli can be made to mutate physiologically as well as morphologically by the application of various stimuli Physiological mutations may conceivably be of tremendous importance in the development of improved strains for fermentation processes

7 Despite natural variation, most strains of Aspergillus when subjected to critical transfer and maintenance under rigorous culture conditions can be kept for many years with constant colony appearance, stable morphology, and dependable biochemical activity

PART II

THE MANUAL PROPER

CHAPTER VII

THE USE OF THE MANUAL

Since the purpose of this manual is to facilitate the identification of Aspergilli as they are isolated from nature and as they are encountered in the investigation of special problems the procedures and considerations involved in the use of the manual must be discussed The general morphology and structural details found in the spore-producing apparatus of the Aspergilli have been described and figured in Chapter III Complexities in the specific combinations of these characters found in the examination of moldy material and in the isolated colonies of individual strains makes desirable a summary outline of the exact observations to be made in describing an Aspergillus Such a descriptive sheet is presented as page 82 For practical use a standard sheet of record paper is folded over on the left hand margin for about 5 cm The column of observations desired is written upon this marginal fold a fresh sheet of paper is slipped under the fold and the descriptive data are filled in appearing exactly in the same order for each strain studied A single glance at the sheet shows the discrepancies if any in the descriptive data obtained With such a sheet properly filled out the keys to groups and within groups facilitate the placement of the strain in its proper group first then its allocation to species within the group

Such descriptive sheets to have comparative value in species diagnoses must present their data in standardized terms It has therefore been necessary to define and illustrate the morphological terms accepted in this manual and to indicate as synonyms in the chapters on morphology the usages of various describers of Aspergilli back over the 200 years since Micheli

Identification from specimens The field mycologist working with specimens collected and examined fresh or dried will often find completion of a technical description very difficult and some observations impossible One with long acquaintance with the Aspergilli may place his specimen to the group or aggregate species correctly but even such workers are frequently puzzled If the organism is deemed important the fresh or recently collected specimen should be taken to the culture laboratory to insure its isolation and preservation in pure form The descriptive data at hand should then be checked and supplemented from the pure culture

To identify an unknown Aspergillus the worker needs pertinent data which will permit him to interpret his mold in terms of species already de-

scribed—including the observations essential in a species characteri For convenience such data may be indicated vertically upon a desc sheet which is elaborate enough to include observations that are re as useful Measurements should be presented as ranges encounte the examination of many units, *not as exact and single measureme* individual cells or structures

Aspergillus—identifying number or marks
Culture medium or natural substratum
Temperature of incubation
Colony characters
- Rate of growth
- Texture
- Mycelium
  - submerged
  - floccose
  - color above
    - reverse

Heads
- color
- form
- measurements

Conidiophore
- length
- diameter
- wall thickness
  - markings
  - color

Vesicle
- shape
- size
- color

Primary sterigmata
- measurements
- *arrangement*
- color

Secondary sterigmata
- measurements

Conidia
- color
- measurements
- markings

Perithecia
- color
- *size*
- shape

Ascospore
- color
- size
- markings

Sclerotia
- color
- size

With such a descriptive sheet before him the user of the manual fi that the Aspergilli have been arranged into a series of natural groups (20) each containing one to several species aggregates Each group cludes species with varieties and at times mutants having a series of sential characters in common These groups have been arranged as nea as possible in natural order, based upon the presence or absence of cert contrasting intergroup characters

These major separating characters are usually evident and positi Nevertheless individual species are found in which certain of these chara ters are reduced to vestigial or apparently suppressed, yet which show many characters allying them with a particular group that such placeme is more logical than any other Such species must sometimes be arbitrari placed and their possible affiliation with other groups indicated both in th discussion of the species and in the discussion of the related group

Species

The species concept in Aspergillus is very difficult to define in tangible terms In this manual the species names already in use have been preserved wherever possible The actual material originally described under a particular species name (i e type material) exists for but a few species If such material exists, it is more important as fixing one point one individual strain in a series of intimately related variants than tying the name to extremely definite morphology If such material is not known comparison of large numbers of strains in pure culture with authoritative descriptive information supplemented by laboratory usages coming down from the original describer usually fixes the series or form intended

From such composite sources it is commonly possible to establish a fairly concrete morphological aspect based upon ranges of color differences in structure, and variations in spore measurement which are repeated in great numbers of isolates In such series of isolates there are no sharp lines of demarcation when large numbers of strains are brought together Within our concept a single strain may show much of this variability within its colonies in culture or it may reduce or suppress certain characteristics and intensify others Such variants have often been given species rank by workers unaware of the existence of other variants completely bridging the gap between such forms and other members of the series Great differences in biochemical activity may be shown by different strains with or without contrasting morphology Nomenclature based upon an assumed correlation of a particular cultural aspect with industrial significance has been offered but has proved utterly unreliable in identifying an organism if lost or in seeking a new strain to serve the same purpose

Two contrasting tendencies in classification are always encountered In the Aspergilli these may be represented by Mosseray (1934a) who found diagnostic marks to distinguish 35 species among 63 cultures of black Aspergilli in the collection of Biourge at Louvain He later received many more variants and faced the question whether to try to describe them all or abandon the field He admitted inability to write descriptions explicit enough to identify them all In contrast Neill (1939) disregarding ascospore measurements and markings lumped ' all of the *A glaucus* group into *A glaucus* Link Likewise all of the black Aspergilli were considered as *A niger* van Tieghem Forms that he did not happen to recognize as belonging to one of his groups were discarded These are extremes

With abundant living material before him the student of the Aspergilli can usually recognize as representative a reasonable number of forms which can be described in tangible specific terms Commonly forms which actually play a significant role in nature or in biochemical processes can be selected as the points around which such species descriptions are drawn

On the other hand forms such as *A janus A itaconicus, A lutescens* etc, while probably rare in nature, possess sufficiently distinctive morphology to warrant species recognition irrespective of other considerations

Varieties

The taxonomic term, variety is used here to designate any homogeneous member of a species complex which carries most of the diagnostic characters of the species but maintains one or more clearly defined differences in particular characters For example variety *alba* is used for certain strains of particular species in which the characteristic color of that species is absent

There is little agreement in the literature in the application of the term variety, certain authors use the term to indicate their belief that one form with particular morphological characters had its origin from another In such cases the belief is hypothetical not a matter of observation Sometimes previously known species were merely moved to varietal standing without specifying the characters upon which the decision was based Such changes are reduced to synonymy or, if entirely unsupported, are occasionally ignored in this manual The term variety is only useful if definitely associated with a clearly defined variation in structures within an otherwise homogeneous series of strains

Mutations, or Mutants

The term mutation, or mutant is only recognized here for forms resulting from a sharp break in morphology (including color) from known structures characteristic of a species, to a definitely altered and inherited contrasting structure Obviously the only excuse for the term in taxonomic usage is to designate the origin of the form studied If the source of such a variant were unknown the taxonomist would designate the form present as a variety or species depending upon the nature and importance of the changes encountered The increasing number of studies in experimental evolution make recognition of induced variation taxonomically necessary

New Species

The discriminating collector will occasionally find an organism markedly divergent in characters from any described form Usually these divergences leave the organism readily recognized as a member of one of the great groups If the differences in aspect and detail of structure separate such a form from the other described members of the group and if the form is found often enough to prove that it has a place in nature description as a new species is warranted Similarly an occasional form either by sup-

pression or complete disappearance loses the arbitrary diagnostic character which furnishes the basis for separating two adjacent groups. In such cases it has at times seemed more practical to add such a species to the group most nearly allied to it by general colony aspect but to cross-reference it to the related group.

The detection and description of species hitherto unrecognized necessitates extensive review of the literature and restudy of available living cultures of at least one whole section of the great genus Aspergillus. Unless the one who encounters a form that he cannot recognize under names already in the literature is prepared to investigate his form adequately in relation to the whole genus, he should not describe his organism as a new species.

Summarized

Comparative study of the taxonomic literature brings out the need for a standardized series of morphological and descriptive terms into which the many usages introduced in the two centuries since Micheli can be translated. Variations in measurement are deemed significant only if they exceed the common limits between closely related organisms and predominate in the preparations examined. Ranges in measurements are more significant than exact dimensions of either selected structures or averaged values based upon many measurements.

Merely quantitative variations are not recognized as warranting separation of species. For example, differences in the shade of color or the intensity of a particular reaction, especially when other strains are found to fall between the old and proposed new species, are not regarded as species characters. Such proposed names either fall to varietal status or to synonymy.

The names not accepted here fall into several categories: (1) Many are listed as synonyms because they are believed to have been given to variants not recognizable by dependable and interpretable differences from other members of the same series. (2) Fantastic variants or monsters appearing in culture may, like Cladosarum, be maintained in the laboratory but unless found perpetuating themselves in nature clearly fall in the class of nature's experiments which do not contribute to the permanent flora. Such names are not regarded as established. (3) Unidentifiable species—names appearing in the literature based upon structures or reactions regarded by the describer as unique but whose identity is so completely lost in large collections among series of closely related strains as to make them unidentifiable by description—are listed in the check list without characterization.

SPECIES KEYS

No general or comprehensive key to the species of Aspergillus is presented Instead, a series of comparatively simple species keys are included *in the discussions of the several groups* The recommended procedure in identifying an Aspergillus with the aid of this manual is first to determine its group relationship by means of one or more of the group keys presented below, then assign the culture more precisely to species by means of the intra group species key for the particular group to which the form belongs

GROUP KEYS

Three keys to the groups of Aspergilli are offered The first is presented in the form of a diagram and presents the different groups in what we con sider their natural order Presentation of this key in graphic form it is believed, will materially assist the user in grasping the various characters which ally and interrelate the different groups Primary separation is based upon the number of series of sterigmata, whether single or double Secondary separation is based upon the character of the conidiophore whether rough or smooth Tertiary separations are based upon the presence or absence of perithecia hulle cells, and sclerotia and upon the color of the conidiophore wall

In assigning species to groups by means of this key, the transitional or intermediate character of certain species becomes strikingly apparent For example *Aspergillus caespitosus* possesses the brown conidiophore and conidial coloration of *A nidulans* furthermore it produces clusters of ir regular, thick walled hulle cells but the head is radiate or only loosely columnar and no perithecia or ascospores are produced It is placed in the *A nidulans* group with full recognition that it possesses certain characters which relate it to *A ustus* *Aspergillus alliaceus* is another form with intermediate characters The conidiophore is uncolored and smooth when examined in liquid mounts (appearing finely roughened when examined dry) and the sclerotia are black but the heads are essentially ochraceous in color It is placed in the *A wentii* group but shows unmistakable relationship to *A ochraceus* *Aspergillus sparsus* is a species of uncertain relationship It possesses a conspicuously roughened yellow conidiophore and globose head, and is placed in the *A ochraceus* group but the conidial heads show a greenish color which is not found in any other known member of this group while the character of the conidiophore will not warrant placement elsewhere The so-called bronze series in the *A tamarii* group is transitional in the direction of *A flavus* Colonies are conspicuously green when young and retain a greenish tint for a considerable period in contrast to *A tamarii* which never shows *true* green and appears greenish only transiently when young Such a list of intermediate species and forms

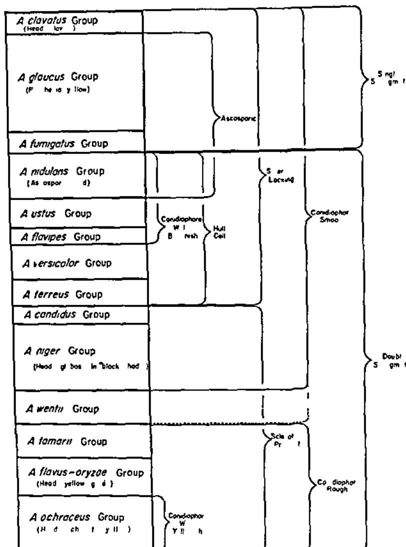


FIG 20 Graphic representation of natural relationships among groups comprising the genus *Aspergillus* The abundance of species in the different groups is roughly indicated by the size of the group boxes in the figure

could be extended but sufficient have been cited to indicate that the various groups like the species which comprise them often cannot be set apart by sharp lines of demarcation

In using this manual, and in studying the Aspergilli generally it is important that the worker should realize that these organisms vary within the species the species vary within the group, and to a lesser extent, the groups themselves vary within the genus In other words, nature did not realize that we were going to write this manual when the various species and groups were being developed, hence not all of the forms one encounters will fit into the various compartments which have been constructed, *although these are on the whole, comparatively elastic This can be illustrated in another way If we completely* disregard color the genus Aspergillus, as depicted in figure 20 can be likened to the spectrum There is a green region and a yellow region in the spectrum and the two regions are, on the whole distinct Furthermore, within each of these certain fixed *and definite lines can be identified It is, however extremely difficult if* not impossible to say where the green region ceases and the yellow begins So it is with the species and groups of the Aspergilli There is a definite *nidulans* group and a definite *ustus* group, but the line separating the two is extremely tenuous

We do not in any sense infer that the Aspergilli cannot be classified—that they cannot be separated into groups, species, and even varieties *This manual is evidence that they can But we do wish to emphasize that* we are dealing with living and variable organisms, and that in describing them, we should be as explicit as possible and still keep our concepts reasonably elastic

Key to Groups—Based Primarily Upon Color

The second key is based primarily upon color and is entirely artificial in its construction The various groups are separated by contrasting coloration and closely related groups may appear widely separated in the key In practice such a key is very useful since color is the most obvious character of an Aspergillus and since the species comprising a particular group with but few exceptions are characterized by variations in shade of color rather than differences in basic coloration Presumptive assignment of the Aspergilli to groups can usually be made from this type of key which is based primarily on color supplemented by *the use of a handlens or dissecting* microscope

A	Conidial heads in definitely green blue green or yellow green shades in young fruiting colonies	B
AA	Conidial heads lacking green colors (Greenish in exceptional cases)	K
B	Conidial heads in green and blue green shades	C
BB	Conidial heads in yellow green shades	*A flavus* group

C	Conidial stalks and heads coarse—heads clavate	*A clavatus* group
CC	Heads not clavate	D
D	Colonies mostly showing yellow perithecia and more or less yellow and red hyphae	*A glaucus* group
DD	Colonies lacking yellow perithecia and more or less yellow and red hyphae	I
E	Colonies producing columnar spore masses	F
EE	Colonies producing radiate globose or hemispherical heads	H
F	Rapidly growing and spreading colonies	G
FF	Slowly and restrictedly growing colonies	*A restrictus* series
G	Conidial columns long narrow	*A fumigatus* group
GG	Conidial columns short and broad perithecia usually present, ascospores red	*A nidulans* group
H	Heads radiate in blue green dull green to pale tan or flesh-colored shades	*A versicolor* group
HH	Heads in some other color	L
K	Heads in long compact columns avellaneous to cinnamon shading toward colorless through light flesh colors	*A terreus* group
KK	Heads in some other color	L
L	Colonies more or less floccose heads in dull olive grays to fuscous	*A ustus* group
LL	Heads in some other color	M
M	Young heads white or only slightly tinged in age	N
MM	Heads in some other color	O
N	Young heads white usually in short columns broadening at apex often becoming avellaneous in age	*A flavipes* group
NN	Heads persistently white larger heads definitely globose or radiate	*A candidus* group
O	Heads in sulphur yellow to ochre shades	*A ochraceus* group
OO	Heads in some other color	P
P	Young colonies showing a greenish color passing into brown	*A tamarii* group
PP	Heads *not showing greenish*	Q
Q	Heads in purple brown to black shades	*A niger* group
QQ	Heads in yellowish brown shades orange to deep brown to umber color	*A wentii* group

Key to Groups—Based Primarily Upon Morphology

The third key is based primarily upon morphology, with colony color employed as an accessory differentiating character. This key is also artificial in construction and is designed to separate the various groups by the simplest and most direct means possible. Groups naturally related may or may not appear in their proper sequence. With the data developed upon the descriptive sheet (p. 82), most of the Aspergilli can be traced to their proper placement in classification schemes by using the following key. Natural arrangement is disregarded and the same group is occasionally reached in different places in the key.

A	Species producing perithecia and ascospores	B
AA	Species not producing perithecia and ascospores	D
B	Ascospores colorless	C
BB	Ascospores purple red	*A. nidulans* group
C	Perithecia white to flesh color enmeshed in a loose net work of colorless hyphae	*A. fischeri*
CC	Perithecia yellow to orange naked vegetative hyphae often showing red to orange granules	*A. glaucus* group
D	Conidial heads cylindrical clavate vesicles definitely clavate	*A. clavatus* group
DD	Conidial heads not cylindrical clavate	E
E	Colonies showing green or greenish color at some stages of development	F
EE	Colonies lacking green color	P
F	Conidiophore wall rough or pitted	G
FF	Conidiophore wall smooth	H
G	Colonies green or yellow green to yellowish	*A. flavus oryzae* group
GG	Colonies greenish brown when young becoming rich brown or umber in age	*A. tamarii* group
H	Sterigmata in one series	I
HH	Sterigmata in two series	K
I	Conidia elliptical to pyriform	J
II	Conidia spinulose 2.5 to 4μ globose chains in compact columns	*A. fumigatus* group
J	Colonies mostly showing yellow perithecia sterigmata usually coarse	*A. glaucus* group
JJ	Colonies lacking perithecia conidia in narrow columns	*A. restrictus* group

K	Conidiophores in yellow brown shades	L
KK	Conidiophores not colored	O
L	Conidial heads definitely green	M
LL	Conidial heads greenish only when young, then in yellow brown shades	*A. ustus*
M	Ascospores produced—purple red in color	*A. nidulans* group
MM	Ascospores not produced	N
N	Colonies showing irregularly clustered hülle cells	*A. caespitosus*
NN	Colonies not showing hülle cells, bright green, spreading	*A. unguis*
O	Conidial area in dull green shades (sometimes partially or completely replaced by tan)	*A. versicolor*
OO	Conidial area in blue greens	*A. sydowi*
P	Conidiophore walls smooth	Q
PP	Conidiophore walls rough	V
Q	Conidiophore walls pale yellow in outer layer, heads white when young, often becoming avellaneous in age	*A. flavipes*
QQ	Conidiophore walls colorless or partially yellow brown near the head	R
R	Conidial chains in solid columns, compact at base	S
RR	Conidial chains radiate at least in the larger and typical heads	T
S	Conidial heads in avellaneous shades	*A. terreus*
SS	Conidial heads flesh color (in pinkish shades)	*A. carneus*
T	Heads white or tardily in yellowish shades	*A. candidus* group
TT	Heads in darker colors	U
U	Conidial heads globose, in purple brown to black (rarely brown or paler)	*A. niger* group
UU	Conidial heads globose, in yellowish, yellow brown and dark brown shades	*A. wentii* group
	Conidiophore walls rough, yellow, heads yellow to ochre	*A. ochraceus* group

CHAPTER VIII

THE ASPERGILLUS CLAVATUS GROUP

Outstanding Characters

Conidial heads clavate[1], large pale blue green
Conidiophores generally coarse, smooth walled, uncolored
Sterigmata in one series
Conidia elliptical smooth comparatively thick walled

Group Key

Conidial structures not exceeding 4 0 mm in length
Aspergillus clavatus Desm
Conidial structures often *1 to 5 or more cm in length*
Aspergillus giganteus Wehmer

Aspergillus clavatus Desmazieres in *Ann Sci Nat Bot (2) 2 71, p 2, fig 4* 1834

Colonies upon Czapek's solution agar growing rapidly at 20-24° C plane or slightly furrowed in certain strains tending to become floccose but generally characterized by a surface mycelial mat and abundant erect conidiophores up to 3 0 mm in length bearing large blue green clavate conidial heads evenly distributed or arranged in more or less well defined zones (Pl III A and Fig 21 A), reverse generally uncolored but becoming browned in age in some strains odor strongly foetid in some strains not pronounced in others Conidial heads clavate large commonly ranging from 300 to 400μ by 150 to 200μ in age splitting into 2, 3 or more divergent columns of compacted conidial chains (Fig 21 B) approximately slate olive in color (Ridgway *Pl XLVII*) Conidiophores *1 5–3 0 mm in* length 20 to 30μ in diameter comparatively thin walled smooth color less gradually enlarging at the apex into a clavate vesicle which is fertile over an area *up to 200 to 250μ in length and 40 to 60μ or more wide* (Fig 21 C) Sterigmata in a single series varying in size from 2 5 to 3 5μ by 2 0 to 3 0μ at the base of the vesicle to 7 0 or 8 0 and occasionally 10μ by *2 5 to 3 0μ at its apex (Fig 21 D)* Conidia elliptical comparatively

[1] *Aspergillus janus* Raper and Thom (see page 187) is characterized by a smaller clavate vesicle in one of its conidial phases It is hardly to be confused with the *clavatus group however because of the whiteness of its* conidial masses the double series of sterigmata and the intermixture of more or less abundant green *A sydowi* like heads in cultures at room temperature

heavy walled smooth 3 0 to 4 0 μ by 2 0 to 3 0 μ occasionally larger in some strains and irregular in others

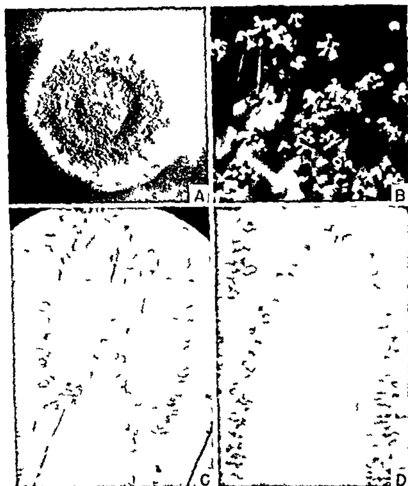


Fig 21 *Aspergillus clavatus* Desm *A* Colony growing on Czapek's solution agar 10 days room temperature × 1 3 (Thom No 5169) *B* Conidial heads showing characteristic splitting in age × 12 (Strain NRRL No 1873) *C* Conidial heads showing characteristic clavate form of vesicle × 180 (Strain NRRL No 6) *D*, Portion of a conidial head further enlarged showing closely packed single series of sterigmata × 600 (Strain NRRL No 6)

Cosmopolitan in distribution and especially common in soil decaying vegetation dung and other materials where active decomposition of nitrogenous materials is taking place Cultures examined include numerous

strains from various parts of the United States together with isolations from China British Guiana, Cuba Panama and European sources

The species description as presented is based upon a large number of closely related strains that have been examined and is adequately represented by such specific strains as NRRL Nos 2 5 8 and others

Upon malt extract agar details of morphology and colony characteristics may or may not conform with those listed above For example, in such typical strains as Nos 2 5, and 8 conidial structures are generally more abundant upon malt than Czapek's agar and may average as much as 20 to 25 per cent larger in size In other strains such as Nos 4 and 6 a markedly different response is noted upon this medium Conidial heads, although



FIG 22 *Aspergillus clavatus* Desm (Strain NRRL No 1 Thom No 107) *A* Colony growing upon Czapek s solution agar 10 days room temperature × 1 3 *B* Conidial heads diminutive and somewhat atypical but showing characteristic clavate form × 600

greatly increased in number are much reduced in size with conidiophores generally 1 mm or less in length bearing heads only 100 to 125µ long and *proportionately reduced in diameter* In these latter forms conidia are somewhat irregular in form and generally larger than in the more typical *strains first considered*

Culture NRRL No 1 (Thom No 107) differs markedly from the species description in producing *deeply floccose colonies (Fig 22 A) and comparatively* few spore heads which are extremely variable in size These range from very small fruiting structures (Fig 22 B) borne as branches upon aerial hyphae to structures arising from the substratum which are characterized by dimensions almost typical of the species This culture is more nearly normal upon malt than upon Czapek s solution agar but is unique

among the strains examined by us and must be considered somewhat atypical It deserves particular attention because it was obtained from the Centraalbureau in Baarn in 1908 as *Aspergillus clavatus* Desm and has undoubtedly been widely distributed by that organization—in fact it is quite probable that it has been examined by more investigators than any other strain belonging to this group We refrain from using it as a basis for the species description however despite its classic history since the less floccose and more heavily sporing strains are so much more commonly encountered in nature

Members of this species produce a strong alkaline reaction upon many culture media and this is usually associated with a strong foetid odor For example when grown upon Czapek s solution agar containing only $NaNO_3$ as nitrogen and sucrose as a carbon source the reaction of typical strains may reach pH 9 5 or even higher accompanied by a strong odor of trimethylamine almost approaching putridity No other species of Aspergillus is known to react in this manner although some strains of *A flavipes* give some suggestion of it The ability to produce and more particularly to withstand strong alkaline conditions undoubtedly accounts for the common occurrence of this species upon dung and other nitrogen rich substrata undergoing decomposition

Aspergillus giganteus Wehmer in Central f Bakt etc 2 Abt 18 No 13/15 385 1907

Colonies upon Czapek s solution agar growing rapidly at 20 C characterized by an extensive surface and submerged vegetative mycelium and an early development of abundant conidiophores 2 0 to 4 0 mm high followed by the subsequent development of less numerous conidiophores ranging up to several centimeters in length (Pl III B and Fig 23 A) the latter strongly phototropic and generally more abundant in marginal areas commonly obscuring the more central mass of short conidiophores colonies at first white becoming pale blue green as conidial heads mature reverse dull tan becoming brown in age odor none to somewhat foetid in certain strains Conidial structures varying greatly in dimensions and falling for the most part into two general size ranges (1) conidiophores commonly 2 to 3 mm rarely exceeding 4 mm in height bearing clavate heads 200 to 350μ in length (2) conidiophores one to several centimeters in length bearing heads up to 1 mm in length The relative proportions of these head types is strongly influenced by environmental conditions and specific strain characteristics Conidial heads pale blue green in age splitting into 2 or more columns extending the length of the vesicle (Fig 23 B) Vesicles consisting of the expanded terminus of the conidiophore ranging from 100 to 250μ by 30 to 50μ upon short conidiophores to 400 to 600μ by 120 to

180μ upon long conidiophores (Fig 23 C) Sterigmata in a single series ranging from 3 0 to 4 0μ by 2 5 to 3 0μ at the base of the vesicle to 6 0 to

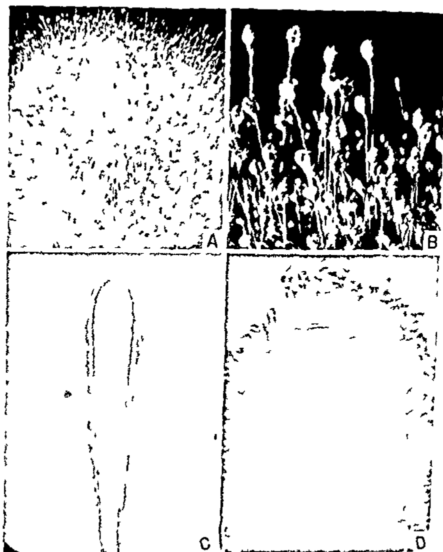
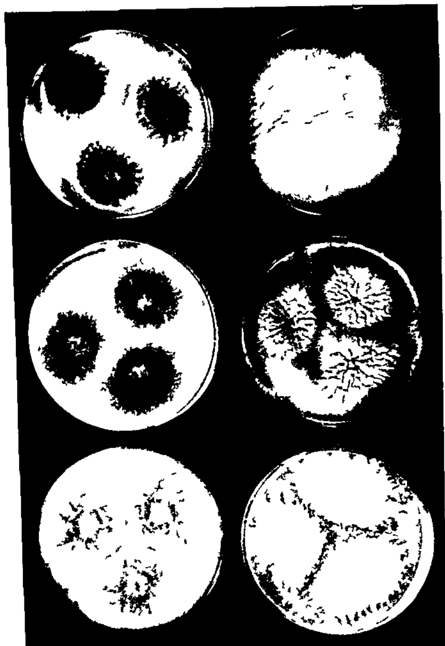


FIG 23 *Aspergillus giganteus* Wehmer (Strain NRRL No 10) *A* Portion of colony on Czapek s solution agar showing characteristic long conidiophores and large clavate heads 10 days room temperature × 1 3 *B* Portion of colony margin somewhat enlarged × 11 *C* Single conidial head showing the very elongate vesicle characteristic of the species × 130 *D* Terminal portion of vesicle showing the closely crowded single series of sterigmata × 600

8 5μ by 2 8 to 3 5μ at the apex (Fig 23 D) Conidia elliptical thick walled smooth 3 5 to 4 5μ by 2 4 to 3 0μ



Pt III

4 (ppe lef) 4 *pe g ll l rat* Desm NRRL N 8 *B* (pper rigl t) *4 pe gillus giganteu* W hme NRRL N 10 hated t 20 C ne-e ded ll m nat C (ce te l ft) *A perg ll pens* (C l) d B y NRRI N 2) *D* (ce te rigl t) *4 perg ll ruber* Brem NRRL N 54 *E* (low left) *4 perg l l t l d m* (M) Th m d Cl rel NRRL N 90 *F* (lo ri h) *A pergillus re glaucus* Thom d Rape NRRL N 12 F g res *A* 1 *B* grow g po ta d d C pek arl t w th 3 perce t *se C* to *F* ro g po C r k arl tio g w th 20 pe ce t rose (Col ph i rap hs by H es N rth R gional Resea h Laboratory Reprod ced thro gh co-operatio f Chas Pfize & Co I)

The above species description is centered upon strain NRRL No 10 (Thom No 5581 13A) isolated from Yucatan caves by Prof F A Wolf (1938) Additional strains examined include isolations from Texas Illinois Mexico, Puerto Rico, and strain NRRI No 1725 (Thom No 138) received from Dr Westerdijk in 1910 as *A giganteus* Wehmer

In the present treatment we include under the species name *A giganteus* Wehmer all strains which produce conidiophores in excess of 1 cm in length While this may appear somewhat arbitrary it is done since members of the *A clavatus* group seem to fall into two natural series (1) those which never produce conidiophores in excess of 5 to 6 mm in length irrespective of environmental conditions or medium composition and (2) those which regularly produce few to many very long-stalked fruiting structures under the usual conditions of laboratory cultivation and examination To the first of these series is applied the species designation *A clavatus* to the second *A giganteus*

Strains of *A giganteus* like those of *A clavatus* differ materially in their growth and cultural appearances upon different culture media More striking however is their response to light and temperature This has been observed by Wehmer (1907) Wolf (1938) and others and has been studied somewhat exhaustively by Webb (1942) Using the Wolf isolate, Webb found that the production of long conidiophores was favored by cultivation upon media containing from 1 to 10 percent sucrose and incubation at 20 C in the presence of light or darkness whereas heavy conidial production and the development of short conidiophores were favored by incubation at 30° C in darkness Different strains vary materially in their cultural appearance upon such standard media as Czapek s solution agar and can be roughly grouped into three sub-series as follows (1) wholly typical strains consistently producing the cultural picture as defined for the species (2) strains producing an unusually heavy crop of short-stalked conidial structures in colony centers followed by the production in marginal areas only of long stalked fruits typical of *A giganteus* and (3) rather sparsely growing strains in which there is a general admixture of short stalked and scattered long-stalked fruits ranging up to 2 to 3 cm in length The latter group is considered as possibly representing atypical and somewhat depauperate strains of the first The second seems to constitute a consistent and fairly well-defined cultural entity but does not differ from typical forms sufficiently to warrant separation as a variety

The validity of the species *A giganteus* has been questioned by some authors Blochwitz (1929) regarded it as a mutation of *A clavatus* and so designated it in his monograph of the Aspergilli His view may be correct It is our belief however that the species should be retained since forms producing the giant conidiophores noted by Wehmer are repeatedly

isolated from nature, and since no evidence has been presented indicating that these larger forms arise directly from the smaller and more common forms While it is true that conditions can be altered so that *A giganteus* cultures suggest *A clavatus*, no one has yet demonstrated that the reverse can be accomplished

Group Synonyms

The following names have been proposed for specimens belonging to this group but without adequate data to warrant recognition as valid species

A clavellus Peck in N Y State Mus Nat Hist Rept 34 49 Pl 2 figs 1-5 1881 Described from cooked squash in New York State No data is presented which would warrant separation of this form from *A clavatus* Desm

A westendorpii Sacc and March in Rev Mycologique 7 149 1885 was listed from cow dung Correctly assigned to *A clavatus* by Lindau in Deutsch Krypt Fl Pilze 8 152 1907

A fusco-cinereus Ellis and Morgan was the name attached to Morgan s jacket No 674 showing a very small clavate aspergillus which has not been collected again hence never cultivated It was probably some member of this group

A pseudo clavatus Purjewitch in Schrift Naturforsch Gesell Kiev 16 (2) 309 pl 12 1900 see also Sacc Syll 16 1028 The organism in culture was reported as having both primary and secondary sterigmata in a small sized clavatus type of head and perithecia with ascospores which were not adequately described Until *somebody finds this organism again and reports its cultivation and more complete* description it will remain doubtful (See Thom and Church The Aspergilli p 100 1926)

Occurrence and Economic Importance

Members of the *A clavatus* group are quite common in soils and decomposing materials characterized by a comparatively high nitrogen content They appear to be common upon the dung of various animals and in the writers experience have been isolated repeatedly from that of chickens While the subject has not been adequately investigated it is probable that the ability of members of this group to withstand strongly alkaline conditions enables them to operate successfully as agents of decomposition in situations where almost all other fungi are eliminated

Antibiosis

Certain strains of *A clavatus* produce substances in the substratum which are capable of destroying *Staphylococcus* and other microorganisms Such activity was first reported by Weisner in March 1942 (Nature 149 p 356) and subsequently by Waksman Horning and Spencer in August of the same year (Science 96 p 202) To the active substance the latter investigators assigned the name *clavacin* and noted that it appeared similar to if not identical with that studied by Weisner (1942) to which the designation

clavatin has since been applied Additional studies by Waksman and his co workers have further defined its action and enlarged the list of bacterial species inhibited (1942b and 1943) Hooper et al (1944) have demon strated the identity of *clavacin* and *patulin* a bactericidal substance ob tained from *Penicillium patulum* by Raistrick and associates (1943) and reported to be of value in the treatment of the common cold

Philpot (1943) reported the production of a penicillin like substance by a strain of *Aspergillus giganteus* In earlier tests by Wilkins and Harris (1942) this strain had been found to produce a substance active against *Staphylococci*

CHAPTER IX

THE ASPERGILLUS GLAUCUS GROUP[1]

Outstanding Characters

Perithecia generally present, yellow, globose to subglobose, thin walled, suspended in networks of red or yellow hyphae

Asci 8 spored, without definite arrangement usually ripening in 2 to 4 weeks

Ascospores lenticular, smooth or rough walled, generally showing an equatorial line or furrow with or without *flanking ridges or crests*

Conidial heads more or less abundant, radiate to somewhat columnar, typically in some shade of green

Conidiophores smooth walled terminating in dome like vesicles

Sterigmata *in one series* rather coarse

Conidia elliptical to subglobose uniformly and characteristically roughened

General Considerations

Aerial hyphae encrusted with yellow orange or red granules are abundant in perithecial areas of most of the strains of the group Both laboratory cultures and naturally moldy specimens frequently show this as their *most conspicuous character one which is readily recognized with the hand* lens In nature, molds of this group appear as patches of green yellow reddish or reddish yellow mold depending upon the relative abundance of conidial heads perithecia and encrusted aerial hyphae and especially influenced by the composition of the substratum

Representatives of the *A glaucus* group are universally distributed in nature and are significant in the incipient spoilage of many organic materials useful to man They occur particularly upon products characterized by a high osmotic tension such as preserves jams cured meats leather goods improperly dried hay moist grain and soft woods stored under humid conditions The classic habitat is improperly dried herbarium specimens

The earliest references to *any* Aspergilli concern representatives of this group, for botanists early encountered them upon herbarium material Micheli in 1729 used the generic name *Aspergillus* (rough head) for the conidial heads characterized by divergent chains of spores commonly present upon such specimens Later in the century Wiggers (1780) pro

[1] Abridged from Charles Thom and Kenneth B Raper *The Aspergillus Glaucus Group* U S Dept of Agr Misc Pub No 426 Washington D C September 1941

posed the name *Mucor herbariorum* for the yellow perithecia found mixed with the Aspergillus heads which he regarded as a different mold In 1809 Link designated the green heads *Aspergillus glaucus* and the yellow perithecia *Eurotium herbariorum* Half a century later DeBary (1854) proved that the Aspergillus heads and Eurotium perithecia were borne upon the same mycelium hence were one fungus Although it could be maintained that the name Eurotium (designating the perfect stage) should take precedence over Aspergillus (descriptive of the conidial apparatus) most recent authors have tended to go back to Micheli and use the name Aspergillus for the whole group because of the obvious relationship of many conidial forms for which no perithecia are known

Laboratory Cultivation

The pattern and size of the ascospore when present is especially significant in describing species of the *Aspergillus glaucus* group Nevertheless the conidial apparatus and the vegetative mycelium of particular subgroups are so important that pure culture under known conditions is always desirable The character of the colony as well as the amount of growth is strongly influenced by the culture medium and it is only upon substrata characterized by a high osmotic tension that typical perithecia and conidial heads are produced It should be noted however that characteristic heads and perithecia normally develop although few in number in situations where less concentrated media dry out rapidly as at the edge of an agar slant Colony comparisons for correct identification can best be made in Petri-dish cultures in which direct observation with the compound microscope is feasible Incubation at 22 to 25 C will permit the development of satisfactory colonies for descriptive study although the optimum for certain species is above or below this range as will be noted in connection with these descriptions and in the general discussion on the influence of temperature on colony growth and development in the genus (p 45) Colony descriptions are based upon 3 week old cultures except as otherwise stated For comparative culture the authors have followed Dale (1909) in using substrata containing high concentrations of sugar The following formula is recommended

Czapek s solution agar with 20 percent of sucrose

Sodium nitrate	3 gm
Dibasic potassium phosphate	1 gm
Magnesium sulfate	0 5 gm
Potassium chloride	0.5 gm
Ferrous sulfate	0 01 gm
Sucrose	200 gm
Agar	15 0 gm
Dist water	1 000 cc

Group Limits and Relationships

Exceptional strains lacking perithecia but presenting conidial morphology clearly belonging to the *A glaucus* group in its strictest sense are occasionally found In addition the *A restrictus* series (*A penicilloides* series of George Smith, 1931) shows conidial morphology clearly related to the group, but differing markedly from the usual types in colony coloration and in the absence of perithecia Thom and Church (1926) considered these latter types as representing intermediate forms between the *Aspergillus glaucus* and *A fumigatus* groups In the present treatment, we include them as non ascosporic and for the most part diminutive forms sufficiently related to the ascosporic species to be included with them in the *A glaucus* group

Certain other groups of the Aspergilli present characters suggesting those of the "glaucus" group considered here The ascospores of *A fischeri* Wehmer (See *A fumigatus* group) and of the *A nidulans* group (which see also Thom and Raper, 1939) in general resemble those of the *A glaucus* group but the yellow perithecia suspended by yellow and red encrusted hyphae do not occur outside of this group

Group Key (Based Primarily Upon Perithecia and Ascospores)

I Perithecia present
- A Ascospores lenticular 6μ or less in long axis
  - 1 Ascospores with convex faces smooth (or nearly so)
    - a Equatorial ridges lacking furrow absent or showing only as a trace — *A repens* series
    - b Equatorial ridges low and rounded furrow broad and shallow — *A ruber* series
    - c Equatorial ridges thin and flexuous crestlike (spore resembling a pulley) — *A chevalieri* series
  - 2 Ascospores with convex faces rough — *A amstelodami* series
- B Ascospores lenticular 6μ or more in long axis — Large spored species or the (*E*) *herbariorum* series

II Perithecia absent
- A Colonies predominantly in yellow orange to brownish shades
  - 1 Heads approximating those of *A repens* — *A argillaceus* Biourge[*]
  - 2 Heads proliferating giving rise to many subheads — *A proliferans* G Smith
- B Colonies in dark green or blue green shades
  - 1 Restricted velvety with short conidiophores and abundant columnar heads — *A restrictus* series
  - 2 Spreading floccose with long conidiophores and globose heads — *A itaconicus* Kinoshita

[*] Culture distributed by Biourge as a new species Believed to represent only a non ascosporic strain of *A repens* hence not recognized as a valid species by the writers (see p 111)

THE ASPERGILLUS REPENS SERIES

Ascospores lenticular, mostly 4 8 to 5 4μ by 3 8 to 4 4μ smooth walled with equatorial area rounded or somewhat flattened and occasionally indented showing a trace of furrow but without crests or ridges

The *Aspergillus repens* series as based upon the ascospore described includes a great number of universally distributed strains which retain some cultural individuality Consequently several of them have been described as species by earlier workers From comparison of a great series of these forms it seems necessary to bring together under the name *A repens* (Cda) DeBary, a very considerable number of forms (some of them regarded as species by others) in which the ascospore is typical for the group and the colony difference falls within lines of quantitative rather than qualitative variation

The following key is offered as a means of separating culturally distinct strains or groups of strains

A Conidial heads large borne above the surface layer of perithecia and enveloping hyphae
  1 Heads radiate long-stalked — *A repens* (Cda) DeBary
  2 Heads columnar short-stalked — *A dierckxii* Biourge
B Conidial heads small enmeshed with the perithecia in a felt of sterile hyphae
  1 Felt orange yellow loose textured radially wrinkled — *A pseudoglaucus* Bloch
  2 Felt yellow buff close textured plane or nearly so — *A profusus* Hann*

Aspergillus repens (Cda) DeBary in Abhandl I Senkenberg Naturf Gesellsch 7 379 1870

Synonyms *A glaucus* var *repens* Cda Icones Fungorum 5 53 Taf II fig 27 1842
A scheelei Bain and Sart Soc Mycol de France Bul Trimest 28 257–262 pl X 1912
A B var *scheelei* Bain and Sart Soc Mycol de France Bul Trimest 28 262–267 pl XI 1912

Colonies upon Czapek s solution agar (3 percent sucrose) restricted plane or somewhat wrinkled forming a rather compact felt (fig 24 A_1) with the marginal area near Scheele s green (Ridgway Pl VI) from developing heads older areas yellow green to greenish gray and enmeshing large numbers of aborted perithecia producing few asco pores normal perithecia found only when such colonies spread over the bare walls of the vessel Reverse in shades of greenish yellow at colony margin to deep maroon or almost black in older areas

* Species name not recognized as valid by authors of this publication

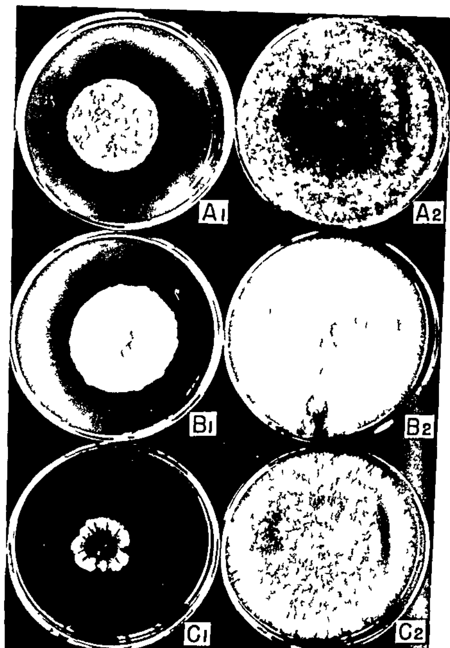


FIG. 24. Comparative growth of *Aspergillus repens*, *A. chevalieri*, and *A. ruber* upon two different culture media; three weeks incubation at room temperature. *A 1* and *2*, *A. repens* NRRL No. 17 upon (1) Czapek's solution agar (3 per cent sucrose) and (2) Czapek's solution agar with 20 percent sucrose. *B 1* and *2*, *A. chevalieri* NRRL No. 78, and *C 1* and *2*, *A. ruber* NRRL No. 52 upon the same media in similar arrangement.

Colonies upon Czapek's solution agar with 20 percent of sucrose spreading broadly and rapidly plane or slightly wrinkled orange yellow commonly characterized by broad zones of dull green conidial heads (Pl III C and fig 25 A and B) surface growth consisting of loosely woven hyphae studded with orange granules enmeshing abundant yellow perithecia above

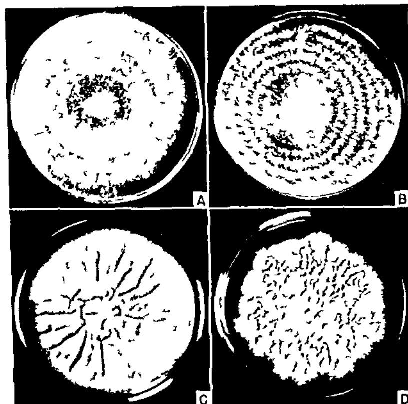


FIG 25 Comparative growth of members of the *Aspergillus repens* series upon Czapek's solution agar containing 20 percent of sucrose incubation at room temperature for three weeks *A* and *B* Typical cultures of *A repens* *C* *A pseudoglaucus* NRRL No 40 *D* *A pseudoglaucus* NRRL No 45

which project abundant conidial heads the whole colony and especially the marginal areas and adjacent wall of the culture dish commonly overgrown by a loose aerial network of hyphae bearing conidial heads and scattered perithecia reverse varying from yellow-orange to deep maroon

Perithecia very abundant borne in loose networks of yellow to orange red hyphae (fig 26 A) yellow spherical to subspherical mostly 75 to 100μ

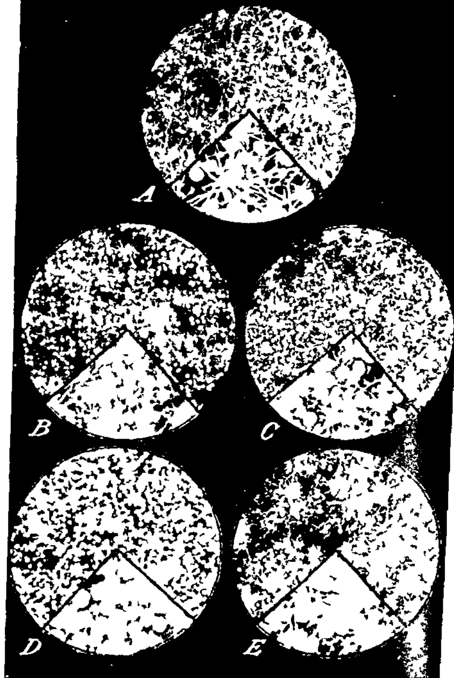


Fig 26 Marginal areas of colonies of representative species of the *Aspergillus glaucus* group showing the relative size and arrangement of perithecia (conidial heads not in focus) *A Aspergillus repens B A chevalieri C A ruber D A amstelodami E A echinulatus* Figures × 10 inserts × 30 (Reprinted from Thom and Raper The *Aspergillus glaucus* Group U S D A Misc Pub 426 1–46 1941)

occasionally up to 125μ asci 10 to 12μ ascospores lenticular mostly 4 8 to 5 6μ by 3 8 to 4 4μ smooth walled with equatorial area rounded or somewhat flattened and occasionally indented showing a trace of furrow but without crests or ridges (fig 27 A) Conidial heads abundant varying in different strains from 125 to 175μ in diameter consisting of diverging chains of conidia radiating from a hemispherical vesicular apex of the conidiophore (fig 28 A), conidiophores smooth mostly colorless 500 to 1 000μ in length broadening at the apex to a vesicular area about 25 to 40μ in diameter sterigmata in one series 7 to 10μ by 3 5 to 4 5μ conidia elliptical to subglobose spinulose mostly 5 to 6 5μ

Represented by cultures NRRL No 12 No 17 and more than a score of others included in this study In this connection it should be noted that of 37 cultures examined in the present study that produced ascospores characteristic of the *A repens* series 29 produced colonies and microscopic details that place them in the species *A repens* as described

This description is manifestly broad enough to include strains approximating the description given by Bainier and Sartory for *Aspergillus scheelei* and *Aspergillus B* var *scheelei* (1912b) Evidently *A scheelei* was thought by the describers to represent a species with somewhat larger ascospores showing a more definite furrow whereas *Aspergillus B* var *scheelei* was a strain with smaller ascospores almost without a trace of furrow Both species were described as characterized by the production of a yellow pigment In the authors experience a distinction based upon color is largely invalidated by variants bridging the whole range from yellow-orange to deep orange red and even shades of brown when large numbers of strains of this series are compared in culture Strains also vary slightly in the pattern of their ascospores some rarely producing spores with a trace of furrow and others bearing a large proportion with such traces But among spores of a single strain limited variation in this character is normally encountered Thus the presence or absence of a slight furrow unless accompanied by significant differences in morphology or colony character would not seem to justify specific descriptions in this series

A strain designated as *Aspergillus dierckxii* presumably by Biourge but thus far unpublished was included in Gould and Raistrick s study of pigment production in the *A glaucus* group (1934) As received from Raistrick s laboratory this organism (NRRL No 39) produces colonies showing no zonate arrangement of conidial heads Further heads are borne on shorter conidiophores than in typical *A repens* and consist of columns of conidia rather than radiating chains Little or no red color appears in the colonies or in reverse Although no other strains showing exactly these differences have appeared in the authors collection separation as a distinct species is believed unwarranted

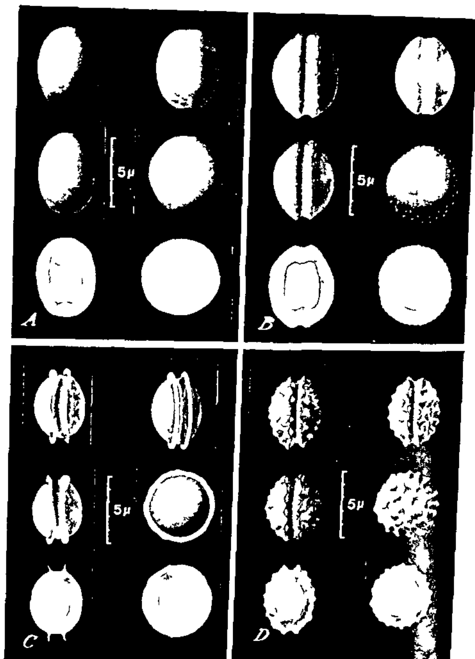


FIG 27 Ascospores representative of the four small spored series of the *Aspergillus glaucus* group A *A repens* B *A ruber* C *A chevalieri* D *A amstelodami* In each species upper left and right and center left spores represent surface profile views center right surface in face view lower left optical section in profile and lower right optical section in face view (Reprinted from Thom and Raper The *Aspergillus glaucus* Group U S D A Misc Pub 426 1–46 1941)

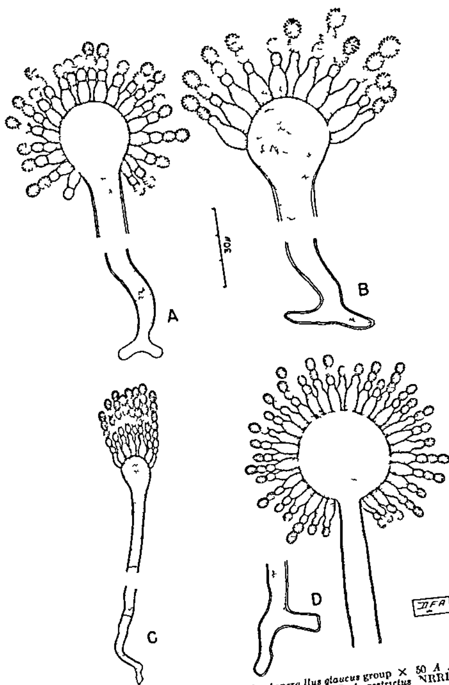


Fig 28 Conidial structures in the Aspergillus glaucus group × 50 *A* *A repens* NRRL No 21 *B* *A echinulatus* NRRL No 131 *C* *A restrictus* NRRL No 154 *D* *A taconicus* NRRL No 161

Aspergillus pseudoglaucus Blochwitz, in Ann Mycol 27 207 1929 emend Thom and Raper, U S D A Misc Publ No 426, p 12 1941

Colonies upon Czapek s solution agar (3 percent sucrose) restricted in growth radiately wrinkled yellow green to shades of gray, consisting of a mixture of small conidial heads, young or aborted perithecia and more or less colorless hyphae reverse orange at center becoming lighter toward the margin

Colonies upon Czapek s solution agar with 20 percent of sucrose spreading strongly wrinkled in a predominantly radiate manner, consisting of a felt of orange-encrusted hyphae enmeshing abundant perithecia orange except at margin where yellow green predominates from the presence of small conidial heads admixed with perithecia in the mycelial felt (fig 25 C), reverse yellow becoming orange brown or maroon in marginal areas

Perithecia abundant, spherical to subspherical, mostly 60 to 80μ though occasionally 100μ in diameter yellow, embedded in a felt of orange mycelium, asci 10 to 12μ in diameter, ascospores lenticular 4 6 to 5 2μ by 3 6 to 4 0μ occasionally 5 6μ in long axis smooth walled with equatorial region rounded or flattened without ridges, and with furrow generally lacking though occasionally showing as a trace Conidial heads few in number and generally submerged in the mycelial felt small mostly 50 to 75μ in diameter but occasionally up to 100μ, conidiophores mostly 150 to 300μ in length 5 to 8μ at the base broadening to a terminal vesicle 12 to 20μ in diameter sterigmata in a single series 6 to 8μ by 3 to 4μ conidia subglobose, delicately spinulose, variable in size ranging from 5 5 to 7 5μ in diameter

Represented in the NRRI collection by No 40 received from Baarn as *A pseudoglaucus* Blochwitz and No 41 received from George Smith as *A fumigatoides* Bain and Sart

There is reason to believe that the former culture is directly derived from Blochwitz s type It becomes necessary therefore to emend the description given by him insofar as the measurements and markings of ascospores and conidia are concerned Gould and Raistrick (1934) reported biochemical data upon a culture No A 38 received from Biourge as *A fumigatoides* Bain and Sart (NRRL No 41) which is identical with *A pseudoglaucus* (NRRL No 40) as sent to the authors by Westerdijk Obviously the culture from Biourge is incorrectly named It does not fit the species description nor the figures of *A fumigatoides* (1909) in the size of its conidia the character of its perithecial wall or the pattern of its ascospores The ascospores of *A fumigatoides* are shown as roughened over their entire surfaces as in *A fischeri* whereas those of No 41 certainly belong in the *A repens* series Distinctive colony characters however maintained stably through many transfers together with the size of the conidial apparatus warrant separating *A pseudoglaucus* from *A repens* and maintaining it as a species

A strain NRRL No 45 received from Dr B O Dodge and Miss Marjorie E Swift of the New York Botanical Garden in 1931 is characterized by an intensely wrinkled colony and a further reduction in the size and number of conidial heads (fig 25 D) Colonies are dull orange red and bear abundant perithecia enmeshed in a close felt of sterile encrusted hyphae Obviously it should be considered with *A pseudoglaucus*

In cultures received from Baarn (NRRL No 44) and from George Smith (NRRL No 45) as *A profusus* Hann (nomen nudum) there is a pronounced accentuation of the floccose habit already noted in *A pseudoglaucus* Upon 20 percent sucrose Czapek agar these cultures which are obviously duplicates produce spreading plane or radiately wrinkled floccose colonies consisting of a close felt of light tan to buff-colored hyphae bearing occasional perithecia and widely scattered conidial heads The perithecia are commonly embedded deep within the felt whereas the conidial heads are most evident at the colony margin Although the ascospores of these strains are definitely of the *A repens* type they are generally flattened along their equators and commonly show a trace of furrow An occasional ascospore shows a minute roughness in the equatorial region The differences observed do not seem to warrant perpetuating the name *A profusus* and in agreement with Dr Westerdijk and coworkers (Centraal bureau List 1939) the cultures have been assigned to *A pseudoglaucus*

Culture NRRL No 46 received from Raistrick in 1923 as *Aspergillus novus* Wehmer (nomen nudum) bears ascospores duplicating those of the cultures just considered This strain is of particular interest, because in routine transfers colonies of two distinct types commonly appear One of these is predominantly floccose and suggests the colonies of the strains received as *A profusus* The other consists of a crowded surface layer of perithecia which is thinly veiled by a loose felt of orange red hyphae and in its gross appearance with the exception of its lighter color, is strongly suggestive of certain cultures of *Aspergillus ruber* The authors agree with Wehmer (1901) and Blochwitz (1929a) that the species designation *Aspergillus novus* should be withdrawn

A nonascosporic culture distributed by Biourge as *Aspergillus argillaceus* n sp was received upon two occasions from Prof Raistrick s laboratory From its appearance in culture and the morphology of its conidial structures this fungus would seem to represent a member of the *Aspergillus repens* series in which perithecial development has been wholly suppressed Although it is questionable whether this fungus represents a true species a brief description is given because of its inclusion in biochemical studies by Raistrick and coworkers (1939 1934 1937) Colonies upon Czapek s solution agar with 20 percent of sucrose spreading irregularly consisting of a loose floccose felt of aerial hyphae and abundant conidial heads pale yellow green to clay color reverse yellow Upon Czapek s solution agar

(3 percent sucrose) colonies restricted, raised in center, thinning toward margin, consisting of abundant conidial heads and interlacing hyphae, buff to clay colored, reverse yellow to tawny Conidial heads abundant dull green, up to 200μ in diameter and commonly splitting into fairly well-defined columns, conidiophores up to 1,000μ in length Conidia subglobose mostly 5 5 to 6 0μ but occasionally up to 7 0μ in long axis, spinulose

ASPERGILLUS RUBER SERIES

Ascospores lenticular, 5 0 to 6 0μ by 4 0 by 4 8μ, colorless with broad, shallow furrow generally evident and flanked by low ridges, and with walls smooth except for minute roughness along the equatorial ridges

This series includes a great number of strains showing variations in cultural appearance but producing ascospores of a limited size range and fairly well-defined pattern For this particular study some 30 strains received from various culture collections and contributors and selected from the isolations made in this laboratory over a period of many years have been chosen for repeated culture and examination Among these, many strains appear distinct but their differences are commonly bridged by intermediate forms Separation within the series, therefore must be along one of the following lines—either (1) strains must be separated upon minor characters, such as differences in the intensity of pigmentation slight variations in ascospore character, etc , or (2) strains must be set off in broad and elastic subgroups in some cases including large numbers which vary appreciably in detail The second alternative is desirable, for the first can lead only to increased hairsplitting and end in greater confusion than that which already exists

Spieckermann and Bremer s designation *Aspergillus ruber* (1902) is assigned to the series, because its members are predominantly producers of an intense red pigment and bear ascospores of the general size and pattern described by these authors The only other described fungus possessing a similar ascospore and characterized by its red color is Bainier and Sartory s *Aspergillus sejunctus* (1911b)

Although it is now quite impossible to say what particular fungus either pair of investigators had at hand both are believed to have worked with members of the large series now under consideration *Aspergillus ruber* is retained since its description is more adequate for the series in addition to being the prior species

To accentuate cultural similarities and differences between the strains studied, a wide variety of culture media has been employed Based upon their appearance in culture the strains fall into a few well-defined subgroups (see figs 29 and 30) The strains belonging to one of these seem

to represent the fungus described by Spieckermann and Bremer (1902) and are at the same time most abundant in the entire series hence are considered as typical of *A ruber* (figs 29 A and 30 A) The general characters of the remaining subgroups are listed to show the extreme cultural variation

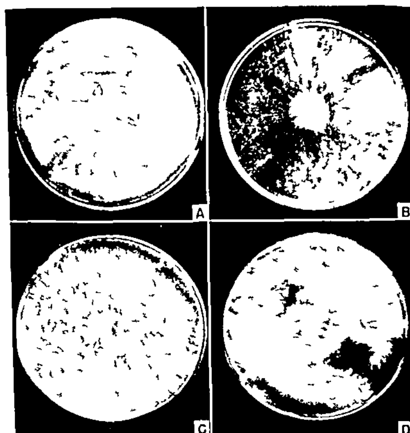


FIG 29 Different colony types developed in the *Aspergillus ruber* series in three weeks at room temperature upon 20 percent sucrose Czapek agar *A* Typical *A ruber* NRRL No 52 *B* NRRL No 70 characterized by thin colonies with mycelium largely submerged *C* NRRL No 65 colony floccose bearing abundant perithecia and few conidial heads and *D* NRRL No 75 deep floccose colony with very abundant conidial heads and only scattered perithecia

that is to be expected within the series but specific names are withheld because to perpetuate or propose such would multiply rather than clarify the confused nomenclature of this abundant and variable series of organisms

To illustrate more definitely the variation that occurs, the following outline of possible lines of separation is inserted

A Colonies predominantly perithecial
  1 Colonies red perithecia abundant in a layer at the agar surface and overgrown by a felt of red encrusted hyphae
    A ruber (Spieck and Brem) Thom and Church NRRL Nos 52 49
  2 Perithecia abundant in a loose floccose overgrowth of red hyphae as well as in a layer at the agar surface NRRL No 65
  3 Colonies thin orange red perithecia abundant in old cultures and on very concentrated media NRRL No 70

B Colonies predominantly conidial
  1 Colonies gray heads long stalked perithecia few NRRL No 75

C Colonies mixed conidial and perithecial
  1 Colonies orange red and green zonate perithecia borne at the agar surface and piled in a loose network of superficial hyphae NRRL No 71

D Colonies predominantly floccose colonies red brown perithecia and conidial heads few *A lovainensis* Biourge [*] NRRL No 76

Aspergillus ruber (Bremer)

Synonyms *A ruber* (Spieckermann and Bremer) Thom and Church in The Aspergilli 112 1926
Eurotium rubrum Bremer in Zeitschr f Untersuch d Nahrung und Genussmittel IV 1901 p 72 also in Die fett verzehr Organismen in Nahr u Futtermitteln Dissert Munster 1902
E rubrum Spieckermann and Bremer in Landw Jahrb 31 81–128 1902
A sejunctus Bain and Sart Soc Mycol de France Bul Trimest 27 361–367 pl XI 1911

Colonies upon Czapek s solution agar (3 percent sucrose) more restricted plane (fig 24 C_1) orange brown to red brown in color perithecia generally abundant though often abortive conidial heads pea green to olive green abundant in some strains few and largely vestigial in others reverse orange red to maroon

Colonies upon Czapek s solution agar with 20 percent of sucrose spreading rapidly and broadly in a regular manner or unevenly, plane predominantly red ranging from ferrugineous to morocco red perithecia very abundant borne in a dense layer at the agar surface and largely concealed within and beneath a close textured felt of red-encrusted hyphae conidial heads projecting above the felt pale gray green to deep olive gray more or less abundant and generally crowded near the center or scattered unevenly

[*] Species name not recognized as valid by authors of this publication

over the colony (Pl III D figs 29 A and 30 A) reverse in shades of dark red brown

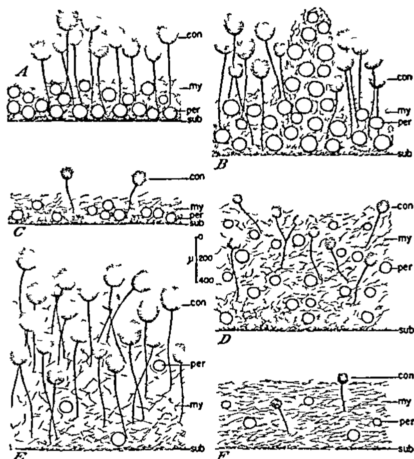


FIG 30 Diagramatic representation of cross sections of different colony types in the *Aspergillus ruber* series developed at room temperature upon 20 percent sucrose Czapek agar showing relative abundance and disposition of the conidial heads (*con*) and perithecia (*per*) and the amount and character of the mycelium (*my*) above the substratum (*sub*) *A* Typical colony of *A ruber* as seen in NRRL No 52 *B F*, atypical colonies as seen respectively in NRRL No 71 No 70 No 65 No 75 and No 76 Scale approximate (Reprinted from Thom and Raper The *Aspergillus glaucus* Group U S D A Misc Pub 426 1–46 1941)

Perithecia very abundant largely enmeshed in a felt at the agar surface (fig 30 A) yellow to orange red spherical to subspherical mostly 80 to 120μ though occasionally up to 140μ in diameter asci 12 to 15μ asco spores lenticular 5 2 to 6 0μ by 4 4 to 4 8μ with furrow generally evident as a broad and shallow depression around the spore equator ridges low and

often inconspicuous walls smooth except for minute roughness along equatorial ridges (fig 27 B) Conidial heads generally abundant, numerous in localized areas or scattered thinly over the colony pale blue green, radiate 150 to 250μ in diameter conidiophore smooth, colorless to orange brown 500 to 750μ in length, broadening to 14 to 16μ where it passes into the subglobose vesicular area of 25 to 35μ diameter, sterigmata in a single series 7 to 9μ by 4 to 5μ conidia elliptical to subglobose, closely spinulose, mostly 5 to 6 5μ in long axis

Aspergillus ruber is represented in this study by NRRL Nos 52 53, and others Of 31 strains examined belonging to the whole series 19 showed colonies and microscopic characters that place them within the species *Aspergillus ruber* as described above Although the majority of strains belonging to the *A ruber* series produce plane colonies as noted in the de scription occasionally strains may produce colonies more or less wrinkled

Culture NRRL No 65 (figs 29 C and 30 D) represents a subseries of several strains that differ from the above not only in colony character upon 20 percent sucrose Czapek agar as indicated in the preceding key, but also in their growth upon media of lower concentration These grow slowly and poorly on Czapek (3 percent sucrose) potato dextrose and wort agars, producing small raised colonies of 1 to 2 cm in diameter bearing neither normal conidial heads nor perithecia

Strain NRRL No 70 (figs 29 B and 30 C) produces abundant perithecia only on very dry areas of the substratum in old cultures or on media con taining a sucrose concentration of 40 percent or more In contrast to other strains, this fungus grows better upon media containing 4 percent agar than the usual 1 2 percent agar further establishing its xerophytic character

Strains such as NRRL No 75 (figs 29 D and 30 E) occasionally en countered, are predominantly conidial and characterized by rampant hyphae bearing abundant conidial heads piled in floccose masses above the substratum and upon the edges of the culture dish or tube They thus produce colonies markedly in contrast with the usual *Aspergillus ruber* concept But the character of their ascospores together with the occur rence of occasional sectors in colonies of these strains showing the usual mixture of perithecia and conidial heads relates them definitely with *A ruber*

Strain NRRL No 71 (fig 30 B) represents a subsection of the series in which conidial heads are abundant and generally arranged in fairly definite zones and patches with loose clusters of perithecia irregularly and conspicuously distributed among and above the grouped green heads These strains are further characterized by somewhat larger perithecia than those of NRRL No 52 being mostly in the range of 125 to 150μ in diameter and by producing less red color in the colonies and in their reverse

Culture NRRL No 76 (fig 30 F) is characterized by a close felt of red brown hyphae which completely covers the agar surface and in which scattered perithecia are borne Conidial heads are scarce and largely confined to the colony margin This strain which was included in Gould and Raistrick s study of pigmentation in the *Aspergillus glaucus* group (1934) was received from George Smith under the name *Aspergillus lovainensis* and attributed to Biourge Except for its dark color this fungus in culture bears a striking resemblance to one received from Baarn as *Aspergillus profusus* (NRRL No 44) which showed similar floccose habits However, the ascospores of the latter are smaller and less furrowed and are essentially smooth along the equatorial margin The degree of relationship between the two is questionable

In addition to the ascosporic strains definitely placeable in the series George Smith has recently described *A proliferans* in which the prolifera tion of the sterigmata has become so pronounced as to become the most conspicuous character while perithecium formation has been suppressed In colony characters however it belongs here This strain diverges further from the type as described but may be arbitrarily placed here by the branching of its simplified heads and the size and markings of its conidia

Aspergillus proliferans George Smith in Brit Mycol Soc Trans 26(1/2) 26 Pl III 1943

Colonies on Czapek s solution agar spreading very slowly with growth at first largely submerged then with matted floccose aerial mycelium white changing to yellowish shades sporing tardily with conidial areas gray green reverse yellowish brown on wort agar growing slowly but better than on Czapek with mycelium white then yellow and finally orange and tardy development of gray green to gray conidial areas becoming more deeply floccose in age especially at shallow end of the slope reverse yellow normal conidial heads loosely radiate conidiophore smooth thin walled usually with one or two septa 4 to 14µ in diameter vesicles occasionally almost globose more frequently obconical or mere broadening of the ends of the conidiophores up to about 20µ in diameter sterigmata when normal in one series 8 to 11µ by 3 5 to 6µ often elongate septate and bearing small secondary heads frequently resembling heads of monoverticillate *Penicillia* or with upper portion much swollen and appearing almost as very large thick walled conidia with long connectives up to 20µ in di ameter with normal and swollen sterigmata often appearing in the same head conidia globose or subglobose rough fairly dark-colored 5 to 9 5µ in diameter perithecia not found (Species description after George Smith)

Aspergillus halophilus Sartory Sartory and Meyer (in Ann Mycol 28(3/4) 36? 363 Pl III 1930) appears from the description based upon colonies grown upon licorice sticks to have been some member of this general group No cultures have been available for comparison hence placement near *A proliferans* of George Smith can be only tentative If placement were to be based upon their figure 1? it might be a species of *Scopulariopsis*

ASPERGILLUS CHEVALIERI SERIES

Ascospores lenticular, mostly 4 6 to 5 0μ by 3 4 to 3 8μ occasionally up to 5 2μ in long axis with walls smooth or slightly rough with crests prominent flexuous often recurved and with furrow conspicuous but consisting more of a trough between extended equatorial crests than a depression in the spore wall

Strains belonging to this series show appreciable difference in colony character and to a limited degree in the surface markings of their ascospores The ascospores of all, however, are characterized by their continuous, prominent equatorial crests which do not form an integral part of the spore wall, but extend well beyond the margin of the spore body proper To use Mangin s exceedingly descriptive term they are characteristically "pulley form "

The following key will serve to differentiate groups of strains within the series

A Ascospore walls smooth
 1 Crests prominent thin flexuous often recurved
 A chevalieri (Mangin) Thom and Church
 2 Crests evident low usually erect
 A chevalieri var *multiascosporus* Nakazawa et al*

B Ascospore walls more or less roughened
 1 Crests thin flexuous often recurved conidia roughened *A oriolus* Biourge*
 2 Crests thicker usually erect conidia smooth
 A chevalieri var *intermedius* Thom and Raper

Aspergillus chevalieri (Mangin) Thom and Church The Aspergilli p 11 1926

Synonym *Eurotium chevalieri* Mangin Ann des Sci Nat Bot (Ser 9) 10 361-362, fig 12 1909

Colonies upon Czapek s solution agar (3 percent sucrose) restricted plane closely felted bluish gray in center with typical heads and perithecia largely confined to marginal area (fig 24 C_1) reverse maroon in center to orange at margin

Colonies upon Czapek s solution agar with 20 percent of sucrose growing best at 30 C or above spreading plane to somewhat wrinkled in central

* Species name not recognized as valid by authors of this publication

area (fig 31 A) with abundant conidial heads in blue green shades distributed evenly over the whole surface or more crowded in localized areas projecting above a layer of abundant perithecia enmeshed in orange red hyphae at the agar surface reverse in shades of orange red to brown more intense in center

Perithecia abundant and closely enmeshed in a felt of orange red encrusted hyphae (fig 26 B) mostly 100 to 140μ occasionally up to 150μ globose to subglobose yellow to orange asci 9 to 10μ ascospores lenticular 4 6 to 5 0μ by 3 4 to 3 8μ with walls smooth with equatorial crests prominent thin and often recurved and with furrow consisting more of a trough between parallel crests than an equatorial depression in the spore body

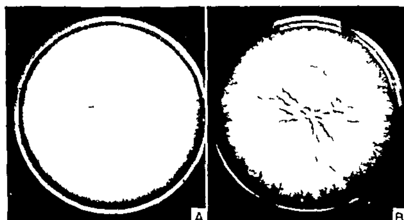


Fig 31 Comparative growth of members of the *Aspergillus chevalieri* series in three weeks at room temperature upon 20 percent sucrose Czapek agar *A* Typical *A chevalieri* NRRL No 78 *B A chevalieri* var *intermedius* NRRL No 82

(fig 27 C) Conidial heads abundant pale blue green appearing radiate from divergent conidial chains mostly 125 to 175μ in diameter occasionally larger conidiophores mostly 700 to 850μ in length enlarging to a vesicular apex somewhat globose 25 to 35μ in diameter sterigmata in a single series closely packed 5 to 7μ by 3 to 3 5μ conidia subglobose spinulose mostly 4 0 to 5 5μ in diameter

Aspergillus chevalieri is represented in the present study by cultures NRRL Nos 78 79 and others The species name is limited to strains bearing ascospores with smooth walls and prominent thin equatorial crests because it is believed that these strains most nearly represent the organism described by Mangin (1909) When grown upon 20 percent sucrose Czapek agar these strains are further characterized by their predominantly orange

red colonies, pale blue green conidial heads and dark colored reverse Within the series different strains vary in the quantity of conidial heads produced, e g NRRL No 79 regularly produces an abundance of heads, NRRL No 78 relatively few

Strains of this series are not so commonly encountered as are those of the *A repens A amstelodami*, or *A ruber* series And within the series strains that conform with the typical species description are relatively less numerous than in these other series In the present study 14 strains belonging to the *A chevalieri* series have been examined and only 6 or less than half, are wholly representative of the species *A chevalieri*

The series as a whole seems to be relatively unstable and certain strains and groups of strains appear transitional between this series and the *A repens* series on the one hand and the *A amstelodami* series on the other

Culture NRRL No 88 received from Baarn as the type of *Aspergillus chevalieri* var *multiascosporus* Nakazawa Takeda Okada and Simo points toward the *A repens* series Its ascospores have smooth walls as in the typical strains of *A chevalieri*, but bear low erect crests in constrast to the thin flexuous crests characteristic of this species Spores lacking crests are occasionally seen and these closely resemble *A repens* The colony upon 20 percent sucrose Czapek agar is definitely of the character of *A chevalieri* Nakazawa and coworkers (1934) separated it from *A chevalieri* because of its more floccose habit and its more abundant production of perithecia The former character is evident in the authors cultures but perithecia are not produced more abundantly than in certain strains entirely typical of *A chevalieri*

Another variation from the typical species is seen in culture NRRL No 87 received from George Smith as *Aspergillus oriolus* and attributed to Biourge The ascospores of this culture (and another that is in the NRRL collection No 81) have crests typical of *A chevalieri* but the spore walls are finely roughened over their entire surfaces This character is suggestive of *A amstelodami* although the roughening of the wall is slight in comparison with that species The colony upon 20 percent sucrose Czapek agar is essentially like that of typical strains of *A chevalieri* but is less red in color and bears fewer conidial heads Although these cultures can be distinguished from type they are not recognized as warranting separation

The roughening of the ascospore wall is further accentuated in a group of four apparently similar strains which it is believed are truly intermediate between *A chevalieri* and *A amstelodami* Because their ascospores bear crests of the *A chevalieri* type and hence appear pulley form they are retained in the species However because they differ from typical strains in additional particulars they are considered a new variety namely *Aspergillus chevalieri* var *intermedius*

Aspergillus chevalieri (Mangin) var *intermedius* Thom and Raper in U S D A Misc Publ No 426 p 21 1941

Colonies upon Czapek s solution agar with 20 percent sucrose differing from the species in texture and color and presenting withal a picture intermediate between *A chevalieri* and *A amstelodami* (fig 31 B) Ascospores lenticular mostly 4.6 to 5.2μ by 3.6 to 4.0μ occasionally 5.4μ in long axis with walls roughened and with prominent equatorial crests *Conidial heads dull green radiate to columnar mostly 100 to 125μ in* diameter and up to 175μ in length conidia elliptical to subglobose smooth walled mostly 3 to 4μ in long axis

Represented in this study by culture NRRL No 82 which was received from George Smith as No 107 and bore the following notation I olated G S from cotton yarn 1927 Close to *A chevalieri*—differs in having smooth small conidia and asco pores somewhat larger than type Duplicated by three additional strains received from European sources

Aspergillus chevalieri var *intermedius* appears to be transitional between the *A chevalieri* and the *A amstelodami* series Such a view is supported (1) by the pattern of the ascospores which shows both the extended and often recurved equatorial crests characteristic of *A chevalieri* and the rough spore walls of *A amstelodami* and (2) by the coloration of the colony *Aspergillus chevalieri* var *intermedius* upon 20 percent sucrose Czapek agar becomes orange yellow above and orange to light brown in reverse *A amstelodami* remains bright yellow with reverse uncolored whereas *A chevalieri* becomes red in the colony and reverse The smoothness of conidia in *A chevalieri* var *intermedius* is a distinctive character and appears in neither *A chevalieri* nor *A amstelodami* Although this variety from many points of view appears to be a hybrid proof of such origin is lacking

Aspergillus diplocystis (Sartory Sartory Hufschmitt and Meyer) Dodge Med Myc p 625 1935 Syn *Eurotium diplocystis* Sartory Sartory Hufschmitt and Meyer in Compt R nd Soc Biol 104 881 883 1930 Not *E diplocystis* B and Br Jour Linn Soc 14 55-56 Tab 10 1875

Characterization Colonies greenish yellow becoming yellow from perithecia Conidiophores erect 50 to 100μ high 3.1 to 3.7μ in diameter membrane thick hyaline Sterigmata confined to a portion of head 5 to 6.75μ by 1.5 to 2.5μ Secondary sterigmata small *conidia spherical 2.75 to 3.1μ in diameter slightly ellipsoid green (tendre to cendré)* sterigmata sometimes abortive and proliferous Perithecia canary yellow asci 4 to 6μ by 5 to 7μ containing 8 ascospores which are ovoid with a furrow and two crests 1.5 to 2.5μ by 1.8 to 3.1μ

This description suggests an Aspergillus with the heads approximating *A nidulans* and the perithecia of *A chevalieri* Ascospore measurements as reported are appreciably smaller than those of *A chevalieri* or any other known species of Aspergillus It was described from a case of onychomycosis from the thumb and the great toe Tentatively placed in the *A chevalieri* series The name is invalid because of *E diplocystis* B and Br 1875

ASPERGILLUS AMSTELODAMI SERIES

Ascospores 4 7 to 5μ by 3 6 to 3 8μ lenticular, colorless with equatorial furrow conspicuous, broadly V shaped and flanked by broad irregular ridges, with walls irregularly and unevenly ridged or roughened over the entire surface

Included in this series are strains that differ greatly in colony appearance However, their close relationship is demonstrated by the similarity in size and pattern of their ascospores and is further shown by the dark olive green color of their conidial heads the bright yellow color of their perithecia, and the absence of any red either in the colonies or their reverse

The following key is designed to show the variation that occurs within the series and to offer a means of separating strains or groups of strains that are culturally distinct

A Colonies predominantly perithecial
  1 Conidial heads abundant in central area and often in concentric zones *A amstelodami* (Mangin) Thom and Church
  2 Conidial heads widely scattered or lacking NRRL No 113

B Colonies predominantly conidial
  1 Perithecia widely scattered superficial NRRL No 111
  2 Perithecia abundant in a felted layer above the conidial heads *A montevidensis* Talice and MacKinnon

C Colonies very thin perithecia and conidial heads widely scattered NRRL No 110

Aspergillus amstelodami (Mangin) Thom and Church, The Aspergilli p 113 1926

Synonyms *Eurotium amstelodami* Mangin in Ann des Sci Nat, Bot (ser 9) 10 360-361 1909
E repens var *amstelodami* Vuill Soc Mycol de France Bul Trimest 36 131 1920

Colonies upon Czapek s solution agar (3 percent sucrose) restricted 4 to 6 cm in diameter plane or closely wrinkled yellow to dull yellow gray in color from abundant perithecia admixed with sterile hyphae and developing conidial heads reverse uncolored becoming tawny in age

Colonies upon Czapek s solution agar with 20 percent of sucrose spreading 8 to 10 cm in diameter more or less wrinkled and zonate (Pl III F and fig 32 A) perithecia very abundant and clustered in masses forming a dense layer at the agar surface (fig 26 D) bright yellow in color lending a characteristic appearance to the colony conidial heads deep olive green abundant in colony center and scattered more or less unevenly over the whole surface, occasionally obscuring the layer of perithecia beneath

Reverse persistently yellow under perithecial areas more or less green where conidial areas predominate

Perithecia globose to subglobose mostly 115 to 140µ in diameter occasionally up to 160µ not covered by or embedded within a felt of sterile hyphae (fig 26 D) asci mostly 10 to 12µ 8-spored ascospores lenticular,

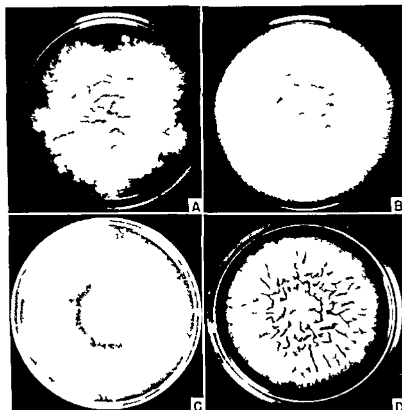


Fig 32 Comparative growth of members of the *Aspergillus amstelodami* series *A* Typical *A amstelodami* NRRL No 90 *B* NRRL No 113 a strain producing abundant perithecia and few conidial heads, *C* NRRL No 111 strain producing abundant conidial heads and very few perithecia *D A montevidensis* NRRL No 108

4 7 to 5 0µ by 3 6 to 3 8µ with prominent V shaped equatorial furrow and broad irregular ridges and with walls roughened over their entire surfaces (fig 27 D) Conidial heads radiate-columnar mostly 120 to 150µ in diameter occasionally larger conidiophores colorless to pale yellow green 275 to 350µ in length broadening to 10 to 12µ in diameter below the vesicle vesicle subglobose 18 to 25µ in diameter sterigmata about 5 to

6 5μ by 2 5 to 3 5μ conidia finely spinulose subglobose, variable in size ranging from 3 5 to 5 2μ mostly about 4μ in long axis

Represented in the NRRL collection by cultures Nos 89, 90 and many others

Thirty two cultures belonging to this series have been examined in the present study Included in this number are the authors' own isolates from a wide variety of sources together with cultures contributed by collaborators in this country and abroad Of these more than three fourths regularly produce colonies conforming with the above description of the species *A amstelodami* Although wide variation in colony character does occur within the series, it is obvious that such variations are exceptional rather than commonplace Accordingly, it is not believed advisable to assign or create specific or varietal names for these variations although they differ markedly from the typical *A amstelodami* in gross appearance An exception to this policy has been made in the case of cultures received as *A montevidensis* for reasons that will be considered later

As indicated in the preceding key to the series marked variation from the normal cultural character of *A amstelodami* occurs along certain divergent lines

Culture NRRL No 113 (fig 32 B) received from Baarn as *Eurotium repens* (Cda) DeBary and Wor var *amstelodami* Vuill (1920) represents a variation that tends toward an almost complete suppression of the conidial phase with only an occasional small and atypical head present

In the opposite direction culture NRRL No 111 (fig 32 C) recently received from Bliss in California (isolated from date fruits) represents a variation that produces a dense stand of conidial heads and only occasional perithecia these being borne above rather than below the layer of crowded conidial heads

In contrast to both of the preceding culture NRRL No 110 isolated from an old shoe produces an extremely thin spreading colony that bears only widely scattered perithecia or conidial heads

A fourth distinct variation is represented by culture NRRL No 108 received in 1932 from Talice as *A montevidensis* Talice and Mackinnon (1931) This fungus is characterized by an initially strong development of the conidial phase and subsequently of perithecia in a felted overgrowth which in the colony center more or less obscures the underlying conidial layer Perithecia and conidial heads are somewhat smaller than in strains of *A amstelodami* Although this culture does not differ from *A amstelodami* more widely than the variations previously noted since it has an imputed pathogenic history and since it has been described and distributed widely under the name *Aspergillus montevidensis* it is believed advisable to retain the name in association with this culture Accordingly the writers include the following emended description

Aspergillus monteuidensis Talice and MacKinnon in Soc de Biol (Paris) Compt Rend 108 1007–1009 1931 emend Thom and Raper U S Dept of Agr Misc Pub 426 p 26 1941

Colonies upon Czapek s solution agar (3 percent sucrose) restricted radiate sulcate with zonation evident toward the margin central area showing coremia perithecia few or lacking reverse and agar very dark almost black

Colonies on Czapek s solution agar with 20 percent of sucrose spreading wrinkled and buckled (fig 32 D) at first bluish green from massed conidial heads with central area later becoming yellow from developing perithecia in a more or less tufted overgrowth of somewhat floccose mycelium reverse in yellow green shades to deep olive in colony center

Perithecia abundant of variable size and irregular shape with relatively few fertile asci and ascospores late in developing commonly 75 to 100μ in diameter occasionally larger asci 10 to 12μ in diameter ascospores lenticular roughened with broad and prominent furrow flanked by low acute and irregular ridges mostly 4 8 to 5 2μ by 3 6 to 4 0μ occasional spores larger or smaller Conidial heads very abundant small somewhat columnar with few conidial chains mostly 70 to 80μ wide occasionally up to 100μ conidiophore up to 300 to 350μ long frequently very short when borne upon the aerial mycelium broadening to a hemispherical dome like vesicular area at the apex commonly deep green or greenish brown vesicle mostly 15 to 20μ in diameter occasionally larger or smaller sterigmata in one series relatively short and thick 6 to 7μ by 3 to 3 5μ conidia roughened subglobose commonly 4 to 5μ by 3 to 4μ occasionally 5.5μ diameter

Type culture isolated by Talice and MacKinnon from the tympanic membrane of the human ear (1931) It is carried in the NRRL collection as No 108

LARGE SPORED SPECIES OR THE HERBARIORUM SERIES

Under *Eurotium herbariorum* Lk Mangin includes all of the members of the group with ascospores more than 6 0μ in long axis (1909) In a general way this represents a very common usage in older literature beginning as far back as Corda in the 1830 s Because neither measurements nor markings of the ascospores were given no one can fix the type of *E herbariorum* In general the species in the large-spored group have both conidia and ascospores definitely larger than those in series already described They become very conspicuous to the collector who finds the anomalous situation of an overabundance of published names and a dearth of isolations Over a period of many years the scarcity of strains isolated in this laboratory which show ascospores larger than 7 0μ leads the authors to believe that such forms are definitely rare if not abnormal This obser

vation is, in effect, confirmed by George Smith (1931) From textiles in particular he has isolated many small spored strains but none with large spores Possibly the present collection contains as many large spored strains as it does because the authors have regarded them as curiosities, and for that reason retained them whereas scores of strains of such common species as *A repens* or *A amstelodami* have been isolated and forthwith discarded

In contrast to the small spored forms where complete duplication between large numbers of isolates is the rule, among the large spored forms there is a marked tendency for each strain to present a somewhat different cultural picture, which is commonly coupled with differences in morphology This would suggest that these forms are unstable and variable, but such a conclusion is refuted by their behavior in culture To illustrate culture NRRL No 131, a strain of *Aspergillus echinulatus*, has for 20 years of continuous culture by the authors retained its distinguishing characters similarly the single known strain of *A medius* has been under observation in this and European laboratories for more than 40 years without appreciable change

Thus the problem of assigning a relatively small number of quite distinct strains is presented To describe each of them would merely add to the confusion already existing hence they have been grouped somewhat, choosing either historic cultures that have become widely distributed or cultures of marked individuality as representing specific names *Homogeneity* among the strains brought together is not claimed The names *A glaucus* and *E herbariorum* are not identified with particular organisms in this discussion

In setting apart a so-called 'large spored series the authors do not in any sense wish to imply close relationship or genetic continuity within this subgroup Species are grouped together primarily as a matter of convenience, and *(E) herbariorum* is selected as the series designation primarily because of Mangin's usage of this species name to cover all of the large spored forms

Key

A Conidial heads green
- 1 Asci ripening within 2 to 4 weeks
  - a Ascospores 6 5 to 7 5μ in long axis — *A mangini* n comb
  - b Ascospores 7.5 to 8 5μ in long axis — *A umbrosus* Bain and Sart
  - c Ascospores 9 0 to 10 0μ in long axis — *A echinulatus* (Delacr) Thom and Church
- 2 Asci ripening slowly 2 to 3 months colonies favored by 40 per cent sugar
  - a Ascospores with equatorial ridges and furrow — *A medius* Meiss
  - b Ascospores usually without equatorial ridges and furrow — *A carnoyi* (Biourge) Thom and Raper

B Conidial heads white — *A niveo glaucus* Thom and Raper

Aspergillus mangini (Mangin) n comb

Synonyms *Eurotium herbariorum* ser *minor* Mangin Ann des Sci Nat Bot (ser 9) 10 365 1909

Aspergillus minor (Mangin) Thom and Raper, U S D A Misc Publ No 426 p 27 1941

Colonies upon Czapek s solution agar (3 percent sucrose) very restricted attaining a diameter of only 1 to 2 cm in 3 weeks irregular and wrinkled cream colored to bluish brown conidial heads present or lacking small perithecia present or lacking mostly abortive reverse uncolored to orange maroon

Colonies upon Czapek s solution agar with 20 percent of sucrose plane or somewhat wrinkled in the central area spreading evenly attaining a diameter of 8 to 10 cm in 3 weeks (fig 33 A) predominantly brick red in color becoming maroon in age perithecia abundant and borne in a close felt of red-encrusted hyphae at the agar surface conidial heads few in number projecting above the perithecial layer generally distributed over the entire colony but occasionally concentrated in localized areas reverse in shades of deep red brown

Perithecia abundant largely embedded in and obscured by a close mycelial felt at the agar surface yellow to orange globose to subglobose mostly 100 to 120μ in diameter occasionally up to 150μ asci 14 to 16μ ascospores lenticular commonly 6 6 to 7 4μ by 5 2 to 5 8μ occasionally up to 7 8μ in long axis finely roughened in the equatorial area ridges low and rounded or pyramidal in section furrow generally definite shallow but often steep sided V shaped

Conidial heads few generally scattered projecting above the perithecial layer pale blue green in color radiate mostly 150 to 200μ in diameter but frequently larger conidiophores smooth pale to dark brown mostly 700 to 800μ in length occasionally reaching 1 mm broadening to 15 to 18μ below the vesicular apex vesicles subglobose 30 to 40μ sterigmata in a single series 8 to 10μ by 4 to 5μ conidia dull green elliptical to sub globose mostly 6 0 to 7 5μ and frequently 8 0μ in long axis

Represented by culture NRRL No 117 isolated from an unpainted board as type and by several additional cultures isolated in this laboratory Culture NRRL No 115 received in 1937 from Oscar W Richards of the Spencer Lens Company differs from the type by producing ascospores of somewhat smaller size and with less evident furrow and ridges Thus it may represent a strain transitional between *Aspergillus mangini* and *A ruber* However it is not sufficiently different from *A mangini* either culturally or morphologically to warrant its description as a separate species or as a distinct variety

Mangin (1909) in his study of the group found specimens in his collec



FIG 33 Comparative growth of large spored members of the *Aspergillus glaucus* group upon 20 percent sucrose Czapek agar at room temperature *A A mangini* NRRL No 117 4 weeks *B A umbrosus* NRRL No 120 4 weeks *C A echinulatus* NRRL No 131 6 weeks *D A niveoglaucus* NRRL No 127 4 weeks *E A medius* NRRL No 125 6 weeks *F A carnoyi* NRRL No 126 4 months (Reprinted from Thom and Raper The *Aspergillus glaucus* Group U S D A Misc Pub 426 1–46 1941)

tion with ascospores less than 7 5μ in long axis yet larger than those of the small-spored forms which he described Having no faith in any of the descriptions existing at the time, he called the aggregate *Eurotium herbariorum* series *minor* The cultures cited above have sufficient common characters to warrant the belief that they are variants of a common stock which may be constituted a species aggregate to which we apply the name *Aspergillus mangini*

Aspergillus umbrosus Bain and Sart in Soc Mycol de France Bul Trimest 28 267-269 pl XII 1912

Probable synonyms *A mutabilis* Bain and Sart, in Soc Mycol de France Bul Trimest 27 458 pl XVII 1911
A mollis Bain and Sart Soc Mycol de France Bul Trimest 27 453 pl XVI 1911

Colonies upon Czapek s solution agar (3 percent sucrose) very restricted attaining a diameter of 0 5 to 1 0 cm in 3 weeks raised tufted white to orange red bearing neither perithecia nor conidial heads reverse colorless to orange brown

Colonies upon Czapek s solution agar with 20 percent of sucrose plane or somewhat wrinkled spreading evenly or irregularly (fig 33 B) reaching a diameter of 8 to 10 cm in 3 weeks predominantly vinaceous red to orange brown in color consisting largely of a surface felt of sterile hyphae encrusted with orange red granules enmeshing abundant perithecia occasionally characterized by a loose floccose overgrowth bearing scattered perithecia conidial heads pale blue green widely scattered and projecting above the perithecial layer reverse in red brown shades

Perithecia abundant yellow to orange globose to subglobose largely embedded in a felt of sterile red-encrusted hyphae at the agar surface occasionally borne in a loose aerial felt mostly 120 to 140μ in diameter rarely up to 175μ asci 14 to 16μ ascospores lenticular mostly 7 2 to 8 0μ by 5 6 to 6 4μ occasional spores up to 8 4μ in long axis finely roughened to smooth in the equatorial areas ridges low and generally rounded furrow shallow commonly V shaped (fig 34 A) conidial heads few scattered projecting above the perithecial layer pale bluish green radiate compact mostly 175 to 250μ in diameter conidiophores smooth colorless to brownish 700 to 850μ in length broadening to 15 to 20μ below the expanded domelike vesicular apex vesicle 25 to 40μ in diameter sterigmata in a single series 10 to 12μ by 4 5 to 6μ conidia pale green elliptical to subglobose spinulose mostly 7 to 8μ in long axis frequently larger

Represented in the present collection by culture NRRL No 120 received in 1925 from Dr Florence A McCormick by European strains and by several cultures isolated by the authors

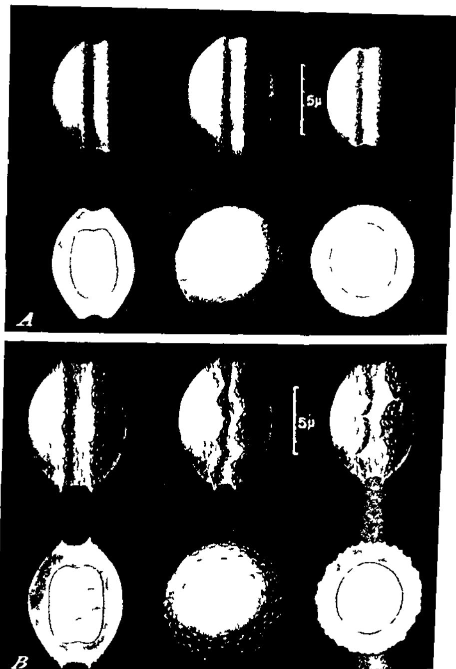


FIG 34 Ascospores representative of *A A umbrosus* and *B A echinulatus* In each species upper left right and center spores represent surface profile views lower left optical section in profile lower center surface in face view and lower right optical section in face view (Reprinted from Thom and Raper The *Aspergillus glaucus* Group U S D A Misc Pub 426 1–46 1941)

Culture NRRL No 123 contributed by Dr Paul Simonart as an unnamed culture from the Biourge collection differs from the species as above described by consistently producing ascospores with walls entirely smooth whereas in other characters the ascospores duplicate essentially those of NRRL No 120 Further NRRL No 123 produces colonies of lighter color than other strains under observation and may in fact represent a fungus comparable to that described as *Aspergillus mutabilis* by Bainier and Sartory

Aspergillus umbrosus *A mutabilis* and *A mollis* were described by Bainier and Sartory (1911c 1912b) primarily upon the basis of colony color (pigment production) and conidial apparatus with the ascopsores of *A umbrosus* recorded as slightly less in long axis (8 0 by 5 6µ) than those of the other species (8 4 by 5 6µ) After careful consideration of the three descriptions and detailed study of the strains in the authors possession showing in general the ascospore described by Bainier and Sartory it is believed that they had at hand three cultural variants of the same species *A umbrosus* is retained as the species designation as it is believed that their description of this species more adequately pictures the cultural and morphological characters of the fungi under consideration than either of the earlier descriptions which are left as probable synonyms

Aspergillus echinulatus (Delacr) Thom and Church The Aspergilli p 107 1926

Synonyms *Eurotium echinulatum* Delacr Soc Mycol de France Bul Trimest 9 266 pl XIV fig III 1893
A brunneus Delacr in Bul Soc Mycol France 9 185 Pl XI fig 9 1893 was described as the conidial stage
E verruculosum Vuill Soc Mycol de France Bul Trimest 34 83 1918

Colonies upon Czapek s solution agar (3 percent sucrose) very restricted 1 5 to 3 0 cm in diameter after 4 weeks marginal area blue green from conidial heads and central portion reddish brown from an overgrowth of sterile encrusted hyphae perithecia lacking reverse deep orange

Colonies upon Czapek s solution agar with 20 percent of sucrose slow growing plane or somewhat wrinkled spreading irregularly attaining a diameter of 7 to 8 cm in 4 weeks (fig 33 C) commonly mottled in appearance due to the uneven distribution of green conidial heads above the underlying orange red perithecial layer conidial heads bottle green abundant commonly crowded in localized areas but scattered thinly throughout the remainder of the colony perithecia abundant and borne in a felt of hyphae encrusted with red granules at the agar surface conspicuous where not obscured by massed green heads reverse cinnamon to deep red brown

Perithecia abundant, embedded in a looose felt of *sterile red hyphae a* the agar surface (fig 26 E) yellow, globose to subglobose mostly 125 t 150μ in diameter, and occasionally up to 175μ, asci 18 to 22μ ascospore lenticular, *mostly 9 to 10μ by 6 5 to 7 5μ occasionally* up to *11μ* in long axis conspicuously roughened in the equatorial area, furrow pronounced broad, ridges prominent and irregular (fig 34 B)

Conidial heads densely crowded in localized areas and scattered throughout the remainder of the colony, bottle green in color, radiate consisting of relatively few, long divergent chains of conidia commonly 250 to 300μ in diameter but often larger or smaller, conidiophores smooth walled colorless to brown shades commonly 700 to 850μ in length, occasionally in excess of 1 mm , broadening from 5 to 7μ at the base to 15 to 20μ below the vesicular apex, vesicle 25 to 35μ in diameter, consisting of a domelike terminus of the broadening conidiophore sterigmata in a single series, not crowded bottle shaped 12 to 15μ by 5 to 7μ, conidia elliptical pyriform, or subglobose echinulate, mostly 8 to 10μ in long axis commonly larger or smaller extremely variable

Represented by NRRL No 131 isolated in 1921 from figs received from California A subculture of this strain forwarded by Miss Margaret Church about 1926, is maintained in the Centraalbureau, the two lines remain identical No ascosporic stage has in the authors experience been found in culture NRRL No 133 received in 1937 from George Smith as *A echinulatus* Delac and obtained by him from Biourge but its conidial *development duplicates* NRRL *No 131 and it is apparently* correctly as signed Da Fonseca s and the Centraalbureau's isolations of *A echinu latus* maintained at Baarn produce somewhat smaller ascospores (8 to 9μ by *6 2 to 7 0μ) and conidia than No 131 but otherwise agree* essentially with the species description as given above Somewhat further reduction in ascospore size is seen in cultures NRRL No 523 isolated from honey, and NRRL No *137 received from George Smith and Raistrick as Asper gillus mongolicus* Biourge (nomen nudum) Although these are less red in color than No 131 and appear distinct in culture the authors do not *feel warranted in separating them as a species or variety believing* that they represent only variations from the general type designated as *A echinulatus*

Bainier and Sartory described *A disjunctus* (1911b) and *A repandus* (1911c) as *vigorous species possessing ascospores 11 by 6μ* and 11 2 by 5 6μ respectively Authentic cultures of these species are not now available but the descriptions as published would seem to place them close to *A echinulatus*

Probable Synonyms

Several Aspergilli with ascospores ranging near that described for *A echinulatus* appear in the literature Unfortunately the details of a co

spore markings are not given and there is a dearth of data to identify them Some of these names are given

A disjunctus Bainier and Sartory in Soc Mycol France Bul 27 346–368 Pl X XI 1911 Ascospores described as 11 2 by 5 6μ with furrow and crests

A repandus Bainier and Sartory in Soc Mycol France Bul 27 463 Pl XVIII 1911 Ascospores described as 11 by 6μ with furrow but no crests

A mencieri Sartory and Flament in Compt Rend Soc Biol (Paris) 83 1114–1115 1920 Ascospores 10 by 4 7μ with furrow and crests

A godfrini Sartory and Roederer in Assn Française pour l Avancement des Sciences 42nd Session Tunis 1913 pp 601–603 1914 Conidial stage only This was growing at blood heat and warmer Its general description suggests affinity with the large spored species of the *A glaucus* group It has not been seen in culture by us

Strains with the particular measurements reported for the three ascosporic species listed above have not come into our collection yet presumably they and many more with minor variations may be found

Aspergillus medius Meiss in Bot Ztg 55 (337)–344 (353)–357 1897

Colonies upon Czapek s solution agar (3 percent sucrose) very restricted 0 5 to 1 5 cm diameter in 6 weeks tufted consisting of a dense growth of yellow brown hyphae bearing neither conidial heads nor perithecia reverse in shades of yellow brown

Colonies upon Czapek s solution agar with 20 percent of sucrose at room temperature very slow growing (optimum 20 C ±) strongly wrinkled tufted irregular in outline attaining a diameter of 5 to 6 cm in 6 weeks (fig 33 E) colony center deep orange red becoming yellow to white at the margin which is characterized by bundles (becoming branching columns at lower temperatures) of hyphae bearing dark green conidial heads in the manner of loose divergent coremia perithecia ripening very slowly, maturing ascospores in 2 to 3 months mostly abortive conidial heads relatively few (more abundant and larger at lower temperatures) and borne either in coremiform masses or scattered throughout the colony reverse in shades of orange maroon

Perithecia scattered mostly abortive borne in a dense felt of orange red hyphae very slowly ripening globose to very irregular in form extremely variable in size rarely attaining a diameter of 125μ containing very few mature ascospores asci 18 to 20μ ascopsores sparingly produced lenticular mostly 8 8 to 9 6μ by 6 0 to 6 8μ occasionally 10μ in long axis somewhat roughened in the equatorial region furrow broad and shallow ridges prominent relatively thin and irregular

Conidial heads deep green radiate compact and of two types Small heads 100 to 150μ in diameter borne on loose coremiform columns and larger heads 200 to 250μ in diameter often scattered throughout the colony

produced more abundantly at 12° to 15° C than at room temperature conidiophores colorless to brown mostly 250 to 350μ in length enlarging to 15 to 20μ below the vesicle, vesicle subglobose mostly 30 to 40μ in diameter, sterigmata in a single series, crowded short 7 to 8μ by 4 to 5μ, conidia green, globose to subglobose, finely echinulate, thick walled mostly 8 to 10μ in diameter, but frequently larger or smaller

Represented in the NRRL collection by culture No 124 which was received in 1924 from Raistrick, who in turn received it from the Centraal bureau It is believed to be Meissner s original strain (1897) Subcultures of this strain are currently maintained at Baarn and by George Smith in London The three lines remain identical as shown by parallel cultures during recent study

This fungus is distinguished particularly by (1) its very slow growth upon 20 percent sucrose Czapek agar at room temperature (2) its tardiness in producing perithecia and especially in ripening ascospores (3) its sparse production of ascospores and (4) its formation of aerial hyphal bundles bearing conidial heads in loose coremiform fashion Further it grows much more rapidly upon Czapek agar containing 40 percent of sucrose than upon that containing 20 percent a difference in concentration which does not materially affect the growth rate of such vigorous species as *A repens* and *A chevalieri* Growth is much more rapid at 20° C than at 28 to 30° C (fig 12) The fungus attains a more favorable form at the lower temperature at which there is a heavier growth of mycelium a more extensive development of aerial hyphal columns and a greater production of conidial heads and perithecia

Culturally this fungus is easily separated from all other species of the group, except possibly *A carnoyi*

Aspergillus carnoyi (Biourge) Thom and Raper, U S Dept Agr Misc Pub 426 p 34 1941

Colonies upon Czapek s solution agar (3 percent sucrose) very restricted reaching a diameter of only 3 to 4 mm in 6 to 8 weeks thin white bearing neither conidial heads nor perithecia reverse colorless

Colonies upon Czapek s solution agar with 20 percent of sucrose at room temperature extremely slow growing (optimum 18 to 20 ( ) reaching a diameter of 7 to 8 cm in 6 to 8 weeks irregular in outline somewhat floccose forming a deep felt bearing abundant perithecia and scattered conidial heads (fig 33 F), orange brown in central area to orange at margin from abundant perithecia in a loose network of sterile hyphae encrusted with orange red granules reverse in orange red shades

Perithecia late in developing abundant yellow to orange globose to subglobose mostly 125 to 175μ in diameter but frequently larger or smaller borne in a loose floccose felt of sterile brown hyphae asci 16 to 18μ

typically 8-spored frequently with some or all spores aborted ascospores lenticular variable in size and pattern mostly 7 2 to 8 4μ by 6 to 6 5μ but often larger (up to 9 0μ in long axis) or smaller (down to 6 5μ in long axis), generally smooth walled but occasionally roughened in equatorial area, generally rounded but often flattened and occasionally indented with ridges wholly absent or indefinite, and with furrow absent or present as a trace only

Conidial heads sparsely produced commonly scattered dull gray green radiate compact mostly 150 to 200μ but often up to 250μ in diameter, conidiophores smooth walled colorless long commonly up to 2 mm in length uniform in diameter 12 to 18μ to just below the vesicle vesicle subglobose 40 to 50μ in diameter and occasionally larger sterigmata in a single series crowded bottle shaped 10 to 12μ by 5 to 6μ conidia globose to subglobose echinulate dull green mostly 8 to 10μ

Species description based upon culture NRRL No 126 received in 1937 as *Aspergillus carnoyi* Biourge from George Smith and by him earlier from Biourge Presumably the culture is type although Biourge s description of the species remains unpublished The culture is distinct, not only differing in its colony character and in the length of its conidiophores but especially in the variable character of its ascospores The majority of spores although much larger resemble those of *A repens* whereas spores with rough walls are occasionally produced This culture is therefore somewhat of an exception to the general rule of constancy in ascospore pattern and the variability of its spores affords one of the best characters for its identification

Aspergillus niveo-glaucus Thom and Raper U S Dept Agr Misc Pub 426 p 35 1941

Synonyms *A glaucus* mut *alba* Bloch Deut Bot Gesell Ber 50 248–256 1932

A glaucus var *albida* Speg An del Mus Nac de Buenos Aires 6 332 1899

Colonies upon Czapek s solution agar (3 percent sucrose) very restricted 1 cm in diameter after 4 weeks white to cream bearing abundant small conidial heads but no perithecia reverse colorless to yellow brown

Colonies upon Czapek s solution agar with 20 percent of sucrose slow growing plane spreading irregularly 6 to 8 cm in diameter after 4 weeks (fig 33 D) thinning toward the margin with mycelium in shades of yellow orange becoming cinnamon brown in age more or less obscured by abundant white heads and with perithecia abundant yellow (Pl III F) embedded and irregularly clustered in a close felt at the agar surface reverse yellow at margin to deep brown at colony center

Perithecia abundant yellow globose to subglobose mostly 100 to 125μ

in diameter occasionally larger commonly clustered borne in an interrupted surface felt of buff to brown hyphae asci 15 to 17μ in diameter ascospores lenticular, mostly 7 2 to 7 8μ by 5 0 to 5 6μ smooth walled

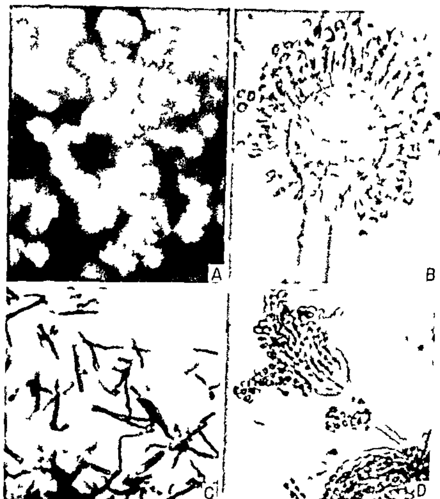


FIG 35 *Aspergillus glaucus* group conidial heads *A* and *B* *A niveo glaucus* NRRL No 127 *A*, surface view showing loose radiate character of heads × 35 *B* photomicrograph of the same showing large globose vesicle fertile over almost the entire surface × 600 *C* and *D* *Aspergillus restrictus* NRRL No 154 *C* surface view showing long columnar heads × 120 *D* photomicrograph of the same showing characteristic small vesicle fertile on the uppermost surface only × 600

except in equatorial area furrows broad and shallow ridges prominent roughened rounded or acute and often appearing ragged Conidial heads abundant white (fig 35 A) often becoming browned in age radiate mostly 250 to 300μ in diameter conidiophores smooth walled colorless to brown,

mostly 1 000 to 1 500μ rarely longer broadening to 16 to 20μ below the vesicle vesicle subglobose 40 to 50μ in diameter (fig 35 B) sterigmata in a single series crowded 8 to 10μ by 3 to 4μ conidia elliptical to pyriform colorless spinulose 6 to 8μ in long axis

The cultural description is based upon NRRL No 127 of this collection as type, this is duplicated by NRRL No 130 received from Baarn in 1938 as *Aspergillus glaucus* Link mut *alba* Bloch Culture NRRL No 128 received in 1935 from George Smith and Raistrick and by them from Biourge as *A albidus* Speg differs from type by consistently producing a greater quantity of conidial heads which are commonly of smaller size the ascosporic stage of the two is indistinguishable Apparently the name 'albidus' is a manuscript use in which Spegazzini s variety (1899) has been raised to species rank presumably by Biourge Blochwitz mutation *albus* assigned to *A glaucus* is untenable because no definite organism can be designated as *A glaucus* Furthermore the specific name *albus* applied to an Aspergillus has already been used in another section of the genus

It is possible that Blochwitz (1932c) is right in regarding this as a mutation but there is nothing to indicate which particular large-spored form is the parent species The strain maintains its identity in culture and hence must be regarded as a species Yuill (1939) in contrast has described white mutants of *A nidulans* and *A fumigatus* and has properly designated them as mutants for they appeared in cultures under observation and are known to have been derived from typical chromogenic strains

THE ASPERGILLUS RESTRICTUS SERIES

Thom and Church (1926) called this series the Intermediate Forms between the *A glaucus* and the *A fumigatus* groups George Smith wishing to emphasize the resemblance of the conidial apparatus to the monoverticillate *Penicillia* called the lot the *A penicilloides* group (1931) thus suggesting Spegazzini s species as the typical member However the important relationship indicated by the structure of the conidial apparatus is not with *Penicillium* but with the *A glaucus* group of which these forms appear to be merely reduced members All of these forms like many of the *A glaucus* forms grow characteristically under conditions of physiological drought—represented by their frequency upon mildewed textiles as studied by Smith (1928) and Galloway (1930) or in concentrated cane products as reported by Owen (1923) Similarly we have found them in many situations in which physiological drought is attained by physical dryness or osmotic concentration attained by the presence of high percentage of sugar or sodium chloride

This natural relationship is on the whole better indicated by accepting Smith s *A restrictus* as typical and regarding the other known members

of this series as allied species To perpetuate an assignment to a subdivision entitled the Microaspergilli, as suggested by some authors would complicate nomenclature without compensating values The series is therefore keyed as a part of the great *A glaucus* group

Series diagnosis Perithecia not found Colonies growing weakly or restrictedly upon Czapek's solution agar, more freely upon wort agar, especially well on high concentrations of sugar or salt, green dark green grayish green to brownish green in various strains and under varying conditions surface growth consisting of conidiophores only or of mycelial felts more or less buckled or heaped, conidiophores smooth slender more or less sinuous, septate, vesicles vary from convex or lens like areas on the broadened apices of conidiophores to definitely ovate to globose enlargements, fertile over all or the upper fraction of such surfaces heads mostly definitely columnar, less commonly radiate hemispherical or almost globose, especially when young, sterigmata in one series, mostly closely packed over the fertile area, varying from 2 to 3μ by 5 to 6μ up to 3 to 4μ by 6 to 10μ conidia barrel shaped to ovate mostly in dark greenish shades, smooth or slowly becoming echinulate or roughened as in the *A glaucus* group commonly adherent into long chains which are packed into columns

Series Key

A Conidiophores broadening upward to produce a convex vesicular apex varying from 8 to 20μ in diameter producing a long slender column of conidia *A gracilis* Bainier

B Conidiophores broadening more abruptly forming a more definite vesicular area
1 Vesicles more convex toward hemispherical
a Slime development evident *A conicus* Bloch
b Slime absent or not conspicuous *A restrictus* G Smith
2 Vesicles ovate to hemispherical columns of conidial chains more or less conspicuous *A penicilloides* Speg

Differences between the above may appear to be of somewhat minor character Nevertheless, each of the sections accounts for sufficient literature to necessitate separate consideration

Aspergillus gracilis Bainier in Bul Soc Myc France 23 92 pl IX figs 11–14 1907

Colonies on Czapek s solution agar very slow growing reaching a diameter of only a few millimeters in several weeks (fig 36A) variously plane or convoluted or buckled with close textured mycelium at first white then slowly green to very dark green with radiating lines of vegetative mycelium about the denser area of the colony growing somewhat better upon wort agar and upon Czapek s agar containing high concentrations of sugar reverse in yellowish shades conidial heads in columns up to 200 or 300μ long

by 10 to 20 even 25μ in diameter straight or twisted Conidiophores mostly arise as very short branches of aerial hyphae up to 20 to 30μ long or less commonly up to 100 to 125μ even up to 250μ and gradually broadening toward the apex to a vesicular area which is very flat dome like almost the effect of a truncated cone 8 10 to 20μ or more in diameter bearing a single series of sterigmata 6 8 10 by 2 to 3μ or in particular strains growing out into little conidiophores producing secondary heads conidia at first barrel form then subglobose about 3μ in long axis in Bainier s strain progressively larger in related strains No perithecia found

The type culture has not been seen Forms with approximately the morphology described however have appeared in strains NRRL No 145 (Thom 4246) from moldy corn Thom 4197.3 (culture lost) from Owen in



FIG 36 *A Aspergillus gracilis* NRRL No 145 on Czapek s solution agar with 20 percent sucrose after incubation for 2 weeks at room temperature *B A restrictus* NRRL No 154 on Czapek s solution agar with 20 percent sucrose after 18 days at room temperature

the sugar laboratory New Orleans and a strain from Thaxter appearing as a contaminant in a *Papulaspora* culture *A gracilis* may thus be assumed to represent a form occasionally found especially in very concentrated substrata Biourge later contributed a culture as type of *A hypojanthinus* originally described by him as *Penicillium hypojanthinum* Biourge (1923) Three other cultures from Biourge labeled *P* (*Microaspergillus*) *hickeyi* *Microaspergillus albo-marginatus* and *P* (*M*) *gueguenı* (figured in his monograph Plate XX but not described) appear in culture to be only minor variations of this general form Later Biourge sent his undescribed *A sartoryi* This grew more freely and showed the conidiophore and vesicle of *A gracilis* conidia were 6 to 7μ or greater in long axis definitely rough and corresponded almost exactly with a culture received from George Smith

as *A gracilis* (NRRL No 156) Whether further accumulation of strains will justify giving Biourge's proposed name *A sartoryi*, sectional or varietal status or merely emphasize the completeness of the series as showing great variations in structural detail is left uncertain There does not appear to be any warrant for preserving *A gracilis* var *exiguus* Bainier and Sartory (1912 a) According to the description this variety differs slightly in physiological characters from *A gracilis* Bainier

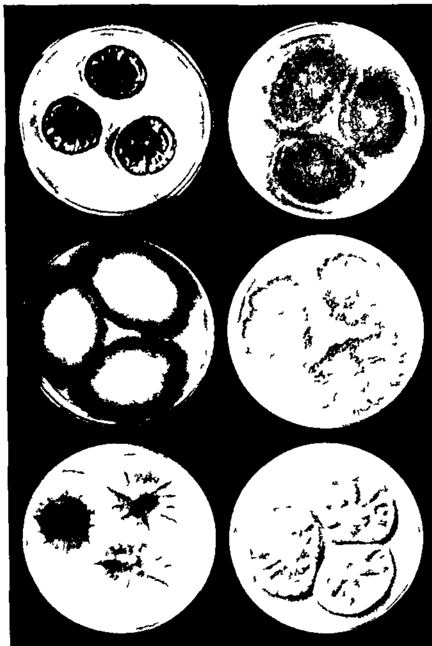
A conicus Blochwitz in Dale Ann Mycol 12 38 1914 Previously described by Dale as a *Penicillium* in Ann Mycol 10 465 1912

See also Thom and Church 'The Aspergilli' p 125 1926

The outstanding character of this species is found by microscopic examination of colonies which become buckled or contorted from a close felting of mycelia Relationship back toward the *A glaucus* group is found in the sterigmata and in the elliptical conidia Heads differing little in microscopic structure from *A gracilis* are found to be more or less completely submerged in dark green to almost black slime Many strains have been collected from widely separated regions showing all gradations from a trace of slime only to complete submergence of the heads particularly in old cultures

Dale isolated the strain first described from English soil and sent duplicate cultures labeled *Penicillium* sp to Blochwitz and to Thom Thom returned the very brief descriptive note without name as published by Dale in 1912 Later Blochwitz proposed by letter to her the name only, *A conicus* which was published by Dale in 1914 without further description Later in his own publication *Die Gattung Aspergillus* (1929) Blochwitz denies the slime development as a character of his organism and redescribes the species in terms to make *A conicus* cover the section of this group represented by Smith s *A restrictus* (1931) Since the name was already in the literature for the slimy series which certainly appears in culture from widely separated places it should stand as originally applied Whether the slime disappears in some strains long kept on artificial media is not settled

In 1928 Biourge contributed under the manuscript name *A cyanogenes* a strain which reproduced the characters given by Thom and Church for *A conicus* This or a nearly related organism appeared as a contaminant in several of the cultures received from Biourge The culture (Thom No 4733 138) figured in his Monograph (1923) as No 126 in Plate XXI under *P glabrum* Dale was another Still another strain from Biourge labeled *A viridans* differed only in the delayed development of the slimy covering of the columnar mass of conidia



PL. IX

A (upper left) *A pe g ll* *t ict* Sm th NRRL N 151 *B* (ppe ght) *A pe gill f g t* Fre se NRRL N 1 3 *C* (ce t left) *4 pe g llus d d* (F l m) W NRRL N 19 *D* (ce t right) *4 per ll varse ol r* (Be k d B Thom l Raper NRRL N 1951 *E* (lo l ft) *A perol us us us* (B Th m d Ch t NRRL N 2 9 *F* (low right *4 pe g llus flavipe* (Ba nd Sart) Th m nd Ch rct NRRL N 2 5 F re *A* gro po C pek sol to agar co ta g 20 pe ce t crose 1 0 5 pe ce t pepto ll tl ul res grow pe ta d d Czapek sol io ga (Colo h tograph b H es N rth Regional Resea h Laborato y Reprod ced thro gh co-operatio f Chas Pfi & Co I)

Neill in his study of the Aspergilli of New Zealand (1939) applied the name *A caesiellus* Saito (1904) to what was manifestly some strain near *A conicus* Blochwitz and then placed the whole group in that species

A restrictus G Smith in Jour Text Inst 22 T115 fig V 1931

Species characterization by George Smith

Colonies growing very poorly on Czapek agar growing moderately well on wort agar dark dull green gradually turning grey or brownish grey reverse in some cultures uncoloured in others green to dark green surface velvety at first, becoming wrinkled and often acquiring a warted appearance (Pl IV A and fig 36 B) heads forming long compact slender columns (fig 35 C) up to 350μ by 20 to 30μ in diameter conidiophores arising mostly from substratum but also as branches of aerial hyphae commonly 50 to 100μ occasionally 150–200μ long by 3 to 3 5μ in diameter often with one or two septa smooth sinuous, uncoloured vesicles flask shaped 7 5 to 14μ in diameter sterigmata in one series borne on upper surface of vesicles only 6 to 9μ by 2 5 to 3μ (fig 28 C) conidia rough spinulose elliptical or somewhat pyriform often showing a distinct connective dark greenish brown 4 to 6 5μ by 3 to 4μ mostly 4 5 to 6μ by 3 to 3 5μ perithecia not found The young conidia are hyaline and cylindrical and almost appear to be segments of enormously elongated septate sterigmata They gradually swell without increasing in length at the same time becoming pigmented but even in old heads they adhere strongly together in columns of parallel chains and mounts (fig 35 D) made in lactophenol usually show compact twisted columnar masses of ripe conidia both attached to and separated from the heads

It is evident from Smith s discussion of a large accumulation of cultures especially from the textile industry that the usual colony type in his experience is not the conicus type with its slime but the columnar head described as *A restrictus* and accepted here as giving the most appropriate name to the series

Among organisms belonging to this series Smith isolated a single strain which differed from typical *A restrictus* in showing somewhat larger dimensions throughout and more particularly in the production of conidia up to 10 μ or more in length and to which he assigned the designation *Aspergillus restrictus* var *B* (G Smith Jour Text Inst 22 T115 Figs IV VI and VIII 1931) Upon examination by us this culture (NRRL No 148) was found to correspond fairly closely to his published description However we do not believe it is sufficiently distinct to warrant continued separation as a variety since other strains showing intermediate dimensions are encountered

Aspergillus penicilloides Spegazzini, in Rev Agrar Veter La Plata, p 246 1896

Spegazzini's description was emended by Thom and Church (The Aspergilli p 126, 1926) then broadened by Smith (Jour Text Inst 22, pp 114–115, 1931) as follows

'Colonies growing fairly slowly on wort agar rich dark green with paler edge, turning darker and duller, and finally becoming dirty greenish grey overgrown with sterile hyphae, reverse brown, greenish brown and dark green in patches, surface much wrinkled and folded heads globose when young 40 to 70μ in diameter becoming columnar, somewhat ragged, and up to 200μ long conidiophores arising either from substratum or from aerial hyphae, smooth, thin walled, 75 to 150μ long by 6 to 10μ in diameter, vesicles rather sharply marked off from conidiophores, pear shaped to subglobose 15 to 23μ in diameter, fertile over the upper half or two thirds sterigmata in one series, crowded, 8 to 10μ by 2 5 to 3 5μ conidia ovate, barrel shaped or nearly spherical usually showing connective rough 3 5 to 5μ by 3 2 to 4μ with very dark colored walls

Thom and Church had strain No 4197 3 isolated from cane products in Louisiana by Owen and agreed to by Spegazzini, NRRL No 151 (Thom No 7) also from cane products fits this description satisfactorily as did also Biourge s strain labeled *A pertardus* Smith reports various strains from mildewed textiles

It would thus appear that the vesicular area in the series varies from the curved apex of a clavate conidiophore as in *A gracilis* to a fairly well defined hemispherical vesicle as in *A restrictus* and finally to an almost globose body as in *A penicilloides* All have so much in common with each other and with the *A glaucus* group that their relationship as degraded mutants appears probable

Aspergillus itaconicus Kinoshita in Botan Mag Tokyo 45 60–61 1931

Probable synonym *A varians* Wehmer in Bot Centralb 80 460–1 1899, also in Wehmer Monogr 77–79 Taf I, fig 1 1899–1901

Diagnosis from Kinoshita s organism obtained from Dr Westerdijk Colonies on Czapek s solution agar forming dense felts 1 to 2 mm deep, white or yellowish ridged and irregular with scattered long-stalked green heads upon dry areas and on the glass, fruiting more abundantly upon malt agar and particularly upon Czapek s solution agar containing 20 percent sugar reverse of colony and agar yellow to orange reddish upon some media heads large light green globose to radiate, breaking up easily under the coverglass conidiophores smooth colorless 8 to 16μ or larger in diameter and up to several millimeters in length under some conditions with

walls 0 5 to 1 5μ in thickness and splitting lengthwise as *A niger* when broken vesicles 15 to 40μ, globose or subglobose (fig 28 D), sterigmata in 1 series 8 to 9μ by 1 5 to 2μ conidia more or less pyriform, 4 3 to 5μ by 3 5 to 4μ finely echinulate of the *A glaucus* type

Diagnosis is drawn from culture NRPL No 161 (No 5344 of Thom) received from Westerdijk as the type of Kinoshita's *A itaconicus* and agrees closely with the description given by Wehmer for *A varians* (cf Thom and Church The Aspergilli p 127 1926) not with Thom and Church's description of their culture No 115 which was subsequently lost *A itaconicus* is so closely related in its physiological responses and in several of its structural characters to the *A glaucus* group that it is placed as an extreme variant at the end of this whole group

Kinoshita described his organism as a producer of itaconic acid (1931a) and detailed his experimental work (1931b) The culture as distributed to laboratories outside of Japan seems to conform to Kinoshita's description and to produce the acid but in quantities too small for commercial development More recently Calam Oxford and Raistrick (1939) have recovered itaconic acid as a metabolic product of *A terreus* From an industrial point of view this source appears to be far more promising

VARIATION IN THE ASPERGILLUS GLAUCUS GROUP

The genetic history of the Aspergilli is an untouched field Separation into large groups is easily made definite enough to include all but a few strains Within these groups variation is so great that differentiation of species requires critical examination and comparison of material including extensive culture Unwilling to undertake this Neill (1939) disposed of the whole group with yellow perithecia by calling them all *A glaucus* On the other hand Mangin (1909), with the same problem of variability before him found the ascospores sufficiently distinctive and dependable to warrant proposing to separate *A amstelodami* and *A chevalieri* as separate species in the *A glaucus* group leaving certain aggregates admittedly inadequately studied Bainier and Sartory (1911b 1911c 1912) working with members of these ill-defined species used color production as the basis for separation They cited ascospore measurements as incidental details in description Because they appear to have had only a few strains in culture and to have described them all as new species the task presented few difficulties to them but unfortunately without the original cultures no one has ever been able to identify their species with confidence Raistrick and his colleagues (1934 1937 1938 1939) using cultures named as received and including an unpublished series from Biourge have given quantitative figures as to pigment production and pigment mixtures for each culture listed by the name on the tube without reporting comparative study of the morphology found

It is clear that wide mycelial or colony variations may be found in nature between strains that retain the ascospore characters of the species. Several such groups have been held by the authors for 30 years or more and furnish convincing evidence that Mangin was justified in using the ascospore as the stable and readily determinable integrating character. Some of these forms retain colony characters in fairly stable form through many transfers on many laboratory substrata and over a period of years. Others grown in various substrata in Petri-dish cultures show sectors or other irregularities in colony habit, which can be picked out and established as strain variants that maintain their special characteristics in continuous culture.

The possibility that variants of similar nature might be induced by chemical stimulation led Thom and Steinberg (1939) to select from the authors' collection certain strains that had remained fairly constant for many years. Of this group they subjected strain NRRL No. 90 of *A. amstelodami* found apparently stable for 30 years to extensive chemical stimulation (1940a). These experiments yielded two groups of effects: (1) A progressive reduction in the production of conidial heads and of perithecia and (2) a great increase in the mass of vegetative mycelia. In no case was spore production completely suppressed although reduced to inconspicuous quantity. The conidia and ascospores when examined were found to have retained the size and markings characteristic for the species whereas the mass of vegetative mycelium became excessive and formed a floccose or cottony mass entirely different in colony appearance from the original.

At this point they reversed the procedure and applied stimulants designed to reestablish spore production (1940b). As a result, the final cultures show abundant green heads with normal conidia and numerous perithecia with ascospores retaining the characters of the species. In routine laboratory examination these extreme variants would not suggest the original strain of *A. amstelodami* although both types of variants produce conidia and ascospores typical of the species.

In 1928, Barnes studied the possibility of heat in inducing variations. He used a strain reported as *Eurotium herbariorum* (Wigg.) Link which had been isolated and maintained in his laboratory for several years without apparent changes. Unfortunately no description of his normal strain was given but a strain received from Westerdijk as Barnes' normal strain proves to be identical with *A. amstelodami* (Baarn strain NRRL No. 89 or strain No. 90 as used by Thom and Steinberg). No reasons were offered for the original identification.

Barnes described a series of experiments in which the spores of his organism were subjected to heat under varied conditions then planted. From the resulting colonies he described 11 variants, 6 of which are available in the Centraalbureau. The authors' transfers of these have been

checked against the descriptions in Barnes paper and obviously represent his isolations

In the following list his designations appear as quotations, followed by our identifications based upon careful cultural study

Flame variant is *A ruber*

Green flame is *A repens*

Blue conidial is *A chevalieri* var *intermedius*

Creamy is a pale yellowish strain of Yuills genus *Cladosarum*

D Brown is *A ustus*

'C Yellow is *A amstelodami*

Of these C Yellow (NRRL No 112) shows the ascosporic pattern and differs little from Barnes normal strain or the authors *A amstelodami* Flame (NRRL No 59) Green flame (culture discarded) and Blue conidial (NRRL No 85) show ascospores of the *glaucus* group but differ markedly in pattern Among large numbers of induced variations changes in the characters of the ascospore have not been found during this study D Brown (culture discarded) produces no ascospores but develops the hülle cells and conidial heads characteristic of the *Aspergillus ustus* group These four forms belong to ubiquitous species quite abundant as contaminants where plant material is handled Such contamination is not satisfactorily excluded by the work reported

Creamy (NRRL No 143) presents a different problem The possibility that this *Cladosarum* was actually derived from the normal *A amstelodami* is not excluded Proliferation of the sterigmata in the head of Aspergilli especially among the *A glaucus* lot is very common The branches produced sometimes are found sterile but usually become diminutive conidiophores with very small vesicles and groups of sterigmata producing normal spores The Yuills *Cladosarum olivaceum* (NRRL No 374) was found as a conspicuous variant or contaminant in their culture of *A niger* (1938) The colony conidiophore vesicle primary sterigmata and initial secondary sterigmata are produced as in *A niger* then instead of chains of conidia with the newest or youngest conidia at the bases of the chains and connected directly with the sterigmata chains of cells are produced that replicate the sterigmata occasionally a chain is interrupted by one cell producing a group of new chains thus acting as a primary sterigma

These chains of cells lengthen not at the base as in *Aspergillus* but at the distal end In spite of prolonged search which shows that the cells toward the outer ends of such chains lose definiteness as sterigmata the authors cannot confirm the finding of a single terminal conidium on each chain as reported by the Yuills This morphological picture is repeated by Barnes Creamy strain which must therefore be interpreted in terms of Yuills genus Only the two isolations are known thus far Their failure to pro

Chapter X

THE ASPERGILLUS FUMIGATUS GROUP

Outstanding Characters

Conidial heads columnar in shades of green through dark green to fuligineus
Vesicles flask shaped, typically fertile over the upper half
Sterigmata in one series crowded
Conidiophores smooth walled usually colored in shades of green
Conidia globose echinulate green

Working with lung material from birds dying of aspergillosis Fresenius, about 1850 described and figured the species *Aspergillus fumigatus* so well that there has never been any doubt as to the morphology of the mold present The investigator of molds in culture however quickly finds that this type of conidiophore and head characterizes not a single pathogenic strain but a multitude of variant forms that are abundant in soil upon decaying vegetation, and in fact wherever organic materials are undergoing even the slightest aerobic decomposition To all of these forms collectively the designation *Aspergillus fumigatus* group is applied

The group falls naturally into two series

Cultures strictly conidial varying from velvety to floccose	*fumigatus series*
Cultures producing perithecia and ascospores conidial development generally limited	*fischeri series*

THE ASPERGILLUS FUMIGATUS SERIES

Aspergillus fumigatus Fresenius in Beitrage zur Mykologie p 81, pl 10, *figs 1–11 Frankfurt, 1850–53 Thom and Church* The Aspergilli p 129 1926

Colonies upon Czapek s solution agar spreading broadly over the substratum *in some strains strictly velvety (Pl IV B and fig 37 A) in others* more or less floccose with varying amounts of tufted aerial mycelium to deep felted or extremely floccose forms (fig 37 B), white at first, becoming green with the development of heads but varying considerably in the final shade of green often becoming dark green to almost black in age Reverse and *substratum in some strains uncolored in others showing varying amounts* of yellow or again passing over in dark red shades in age Conidial heads columnar compact varying in measurement from strain to strain up to 400 *by 50μ but usually much shorter occasionally very small* Conidiophores

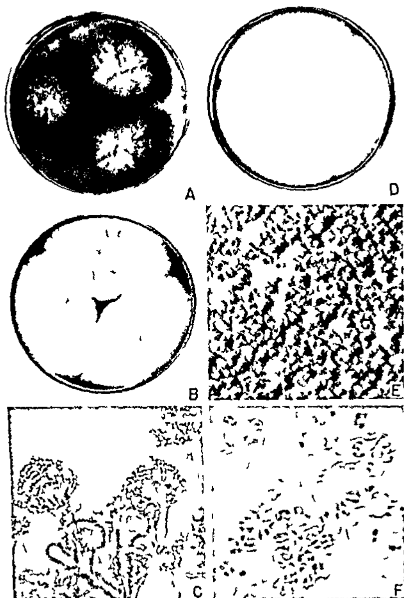


FIG 37 *Aspergillus fumigatus* group *A–C*, *A fumigatus* *A* Typical heavy sporing strain NRRL No 178 on Czapek s solution agar 10 days room temperature *B* Floccose light sporing strain of same species NRRL No 171 and *C* Photomicrograph of typical conidial heads showing characteristic form of vesicles and crowded sterigmata in a single series × 500 *D–F* *Aspergillus fischeri* *D* Strain NRRL No 186 growing upon Czapek s solution agar characterized by very abundant perithecia and few conidial heads *E* Portion of a colony enlarged showing crowded perithecia more or less obscured by loose enveloping hyphae × 6 0 and *F* Asci containing ascospores at various stages of maturity × 500

short smooth, usually densely crowded up to 300μ (in occasional strains up to 500μ) in length by 2 to 8μ in diameter frequently more or less green colored, especially in the upper part, arising directly from submerged hyphae or as very short branches from aerial hyphae septate or unseptate gradually enlarging upward and passing almost imperceptibly into the apical flask shaped vesicles Vesicles up to 20 to 30μ in diameter usually fertile on the upper half only (fig 37 C) Sterigmata in one series usually about 6 to 8μ (varying from 5 to 10μ) by 2 to 3μ crowded closely packed with axes roughly parallel to the axis of the conidiophore Conidia dark green in mass echinulate globose mostly 2 5 to 3μ in diameter with extremes ranging from 2 to 3 5μ Sclerotia or perithecia are not found The species grows well at temperatures up to 45° C or even higher, and is commonly present in compost and other material undergoing decomposition at high temperatures

The species description presented is a composite rather than an exact citation of detailed data about one strain but NRRL No 163 (Thom No 118) may be considered typical Organisms coming within this series as described are world wide in distribution and omnivorous in habit They are regularly abundant in soil and in decomposing organic mas es they are recoverable from apparently sound cereals corn oats wheat etc , they are encountered as pathogenes in the air passages of birds and occasionally as lung parasites of mammals including man One strain (NRRL No 164) was named *A cellulosae* by Hopffe (1919) because of its ability to break down cellulose but when examined it presented no morphologic differences from hundreds of common isolates from soil

Efforts to induce perithecium formation by the conidial strains of *A fumigatus* have been disappointing Organisms maintained in culture over long periods and subjected to multitudes of transfers upon all sorts of substrata have failed to give any response suggesting perithecium formation

Extremes of variation in different strains range from colonies characterized by crowded conidiophores rising vertically from submerged hyphae to a height of 300μ or perhaps at times 500μ then producing columns of conidia sometimes up to 400 by 50μ to very floccose forms in which spore formation is generally retarded and in which conidiophores develop as very short branches of aerial hyphae and produce short columnar heads Many of these variants can be isolated and maintained in culture thus they have been made the types of species by earlier workers Others encountered upon unique substrata have been given specific names in the belief that the substratum relation was obligate Yuill (1939) isolated a buff-colored mutant from a typical green strain and applied to it the designation *A fumigatus* var *helvola* (fig 17 C) Shortly thereafter Steinberg and Thom (1940) likewise recovered an essentially uncolored mutant from a typical green strain Both forms still remain unchanged in culture

If the taxonomist could seize a half dozen widely spaced variants and destroy those which bridge the gaps between identification as separate species would be easy. This however becomes impossible when large numbers of isolates are cultivated and studied in comparative culture. In attempting to divide this great series into tangible entities earlier workers have created a long list of species and while we do not consider these to be valid they are presented in alphabetical order with the places of description. It is believed that all of these should be regarded as synonyms of *A fumigatus* Fres

A ararius Peck in N Y State Museum Rept 44 p 25 pl 4 figs 9-12 1891

A bronchialis Blumentritt in Ber Deutsch Bot Ges 19 442-446 II 22 figs 1-6 1901 also ibid 23 419-427 Pl 19 figs 1 3 6 7 8 19 23 1909

A calyptratus Oudemans in Arch Neerl Ser II 7 283 Tab XIII 1902

A cellulosae Hopffe in Centralb f Bakt etc Abt 83 531-537 1919

A desseyi Spegazzini in Physis (Rev Soc Argentina Cien Nat) VIII 115-117 1 figure 1925 review only seen Rev Appd Mycol 4 542 1925

A fumigatus var *alpha* Sion and Alexandrescu in Compt Rend Soc Biol (Paris) 64 288-289 1908

A fumigatus var *minimus* Sartory in Bul Acad Med Paris 3 Ser 82 304 305 1919

A fumigatus var *tumescens* Blumentritt in Ber Deut Bot Ges 23 419-427 Pl 19 figs 5 6 18-21 1905

A glaucoides Spring in Bull Acad Sci Belg 19 560-572 1852

A lignieresi Cost et Lucet in Ann Sci Nat Ser 9 II 119 tab 5 fig 18-23 1905

A nigrescens Robin in Histoire Naturelle des Vegetaux Parasites p 518 Paris 1853

A pulmonum hominis Welcker in Kuchenmeisters Parasiten II p 144 This is discussed and figured by Theodor von Dusch in Virchow s Archiv (n f 1) 11 561-566 1857 but no ground is given for separating it from *A fumigatus*

A ramosus Hallier in Zeitschr Parsit 2 266-269 pl 6 figs 1-6 1870

A syncephalis Gueguen in Les Champignons parasites de l homme et des animaux 299 pp 12 pl Paris 1904

A virido-griseus Cost and Lucet in Ann Sci Nat Bot IX 2 140 1905

THE ASPERGILLUS FISCHERI SERIES

Aspergillus fischeri Wehmer in Centralb f Bakt etc 2 abt 18 390-2 figs 1-5 1907

Synonyms *A fumigatus*—a cosporic see Thom and Church in Am Jour Bot 5 91-92 1918 See also Thom and Church The Aspergilli p 132 1926

Sartorya fumigata Vuillemin in Compt Rend Acad Sci (Paris) 184, No 3 136-137 1927

The conidial form is strikingly similar to *Aspergillus fumigatus* Colonies grow well upon Czapek s solution agar with conidial heads sparingly pro

duced at room temperature (fig 37 D) but more abundantly at 37° C Conidial heads frequently small and generally of a lighter green color than those of typical *A fumigatus* Perithecia quickly and abundantly produced in most strains dominating the colony appearance (fig 37 E) commonly up to 300μ in diameter, not colored or very pale salmon with walls scarcely colored consisting of a single layer of cells crushing easily covered by a loose network of uncolored sterile hyphae Asci abundant,

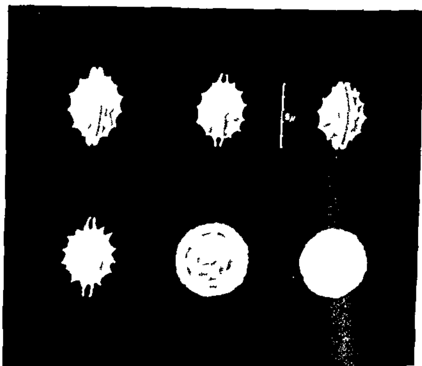


FIG 38 Ascospores of *Aspergillus fischeri* NRRL No 181 (= Thom No 4651 ?) Upper left center and right represent surface profile views lower left optical section in profile lower center surface in face view and lower right optical section in face view

8 spored filling the perithecium within a few days 8 to 10μ by 10 to 12μ subglobose (fig 37 F) breaking down quickly to leave the perithecium full of ripe ascospores Ascospores biconvex uncolored usually about 7 by 4μ consisting of a central body 5 by 4μ with two frilled equatorial banks about 1μ in width roughened with echinulations or anastomosing bands on each convex surface (fig 38) separating into two valves in germination

Culture NRRL No 181 (Thom No 4651 2) received as type from Wehmer in 1923 is apparently identical with numerous isolations from American sources Many strains have been seen including a series from sputum of human cases showing lung involvement by X ray examination

It is regarded as world wide in distribution but seemingly not abundant anywhere. The species grows well at temperatures of 37° C and higher.

Some additional perithecial forms have been described with characters suggesting relationship with *A. fischeri*. However these are inadequately known in culture and assignment here must remain somewhat provisional.

A. malignus Lindt in Arch. Exp. Path. Pharmakol. 25: 257, 271, fig. 1-11. 1889.
Lindt probably had before him some strain which corresponded closely with Wehmer's *Aspergillus fischeri* despite the fact that his description of the conidial apparatus offers sufficient contrast as to lead to question.

A. fumigatoides Bainier and Sartory in Bull. Soc. Myc. France 25: 112, pl. 5. 1909.
While the describers believed this strain close to *A. fumigatus*, Thom and Raper (1941) found it necessary to place the organism studied under this name by Gould and Raistrick (1934) in *A. pseudoglaucus*, although the original description and figures of Bainier and Sartory probably represented material close to or identical with *A. fischeri*.

Sartorya fumigata (Fres.) Vuill. in Compt. Rend. Acad. Sci. (Paris) 184(3): 136-137. 1927.
Sartory, Sartory and Meyer (1926) reported that a culture of *A. fumigatus* subjected to radiation produced an ascosporic form. Later this was designated by Vuillemin (1927) as a new genus *Sartorya*. Neither Vuillemin nor Sartory, Sartory and Meyer appear to have known the relation between *A. fumigatus* and *A. fischeri* although it was pointed out by Thom and Church in 1918. Since the material named *Sartorya* does not appear to have been distributed or fully described, *Sartorya fumigata* (Fres.) Vuillemin may be regarded as a synonym for *A. fischeri* Wehmer and the generic name dropped.

It is believed probable that *Aspergillus fischeri* Wehmer represents the primary organism of this group and that from it the conidial form *A. fumigatus* developed as a species lacking entirely the ascosporic phase. The fact that *A. fumigatus* was described first merely reflects the much greater abundance of this species.

Occurrence and Economic Importance

Aspergillus fumigatus is an extremely cosmopolitan mold and occurs with particular frequency in soil containing appreciable organic materials, upon vegetable matter undergoing slow decomposition, and upon imperfectly dried stored grains. The mold is an important agent in many decomposition processes, particularly at temperatures above 37° C. Growing successfully at 45 to 50° C within the lower reaches of thermophilic decomposition, it is able to operate within a range where most fungi are excluded. Whereas some forms have been described as very active agents of decomposition (e.g. *A. cellulosae* of Hopffe 1919), their more significant role is believed that of forerunners of active bacterial decomposition on the one hand and as slow destroyers of more resistant tissues on the other. *Aspergillus fischeri*, though much less abundant, may be found in situations generally similar to those yielding *A. fumigatus*.

Pathogenesis

There is an extensive pathological literature which covers the occurrence of *A. fumigatus* in lesions of birds and mammals including man together with biochemical and animal experimentation. Such experiments have repeatedly proved that the organism is pathogenic to fowls confined in congested quarters in which moldy grain, straw, and other plant remains are abundant. Direct inoculation to the cornea in laboratory animals causes lesions characteristic for the species.

Infection of human beings occasionally appears, and observations seem to indicate that the patients generally have been exposed to air carrying large numbers of spores. Allergists have reported asthmatic conditions arising from sensitization to this species and Bernton (1930) reports having successfully treated a patient by means of an extract prepared from the spores and mycelium of *A. fumigatus*. The occurrence of *A. fischeri* in cases grouped with *A. fumigatus* is clear indication that the pathogenic principle, whatever it is, is generally present in the group although it may vary in its intensity among different strains as indicated by workers such as Costantin and Lucet (1905).

Among organisms known to be or believed to have been pathogenic strains of *A. fumigatus* the following named forms may be cited: *A. gratioti*, *A. malignus*, *A. fumigatoides*, *A. viride-griseus*, *A. bronchialis*, *A. glaucoides*, *A. nigrescens*, *A. pulmonum hominis*, *A. ramosus* and *A. cellarius* (see p. 151). For a more complete discussion of this group in relation to disease in birds and mammals the reader is referred to Thom and Church's *The Aspergilli* (1926) and Dodge's *Medical Mycology* (1935).

Antibiosis

Anslow and Raistrick (1938a) reported the production by *Aspergillus fumigatus* of a substance to which they applied the name *fumigatin* and in the same year (1938b) reported the species to produce a second metabolic product termed *spinulosin* which they had previously isolated from *Penicillium spinulosum*. In 1942 fumigatin was further discussed by Oxford and Raistrick as a powerful agent against such bacteria as *Bacillus anthracis*, *Escherichia coli*, *Salmonella typhi murinum*, *Staphylococcus albus*, *S. aureus*, *Streptococcus viridans* and *Vibrio cholorae*. Also in 1942 Waksman, Horning and Spencer reported an antibiotic substance termed *fumigacin* to be produced by *A. fumigatus* and presented methods of differentiating this from fumigatin as studied by Raistrick and associates. Fumigacin was found to be both bactericidal and bacteriostatic in its action and to be effective in fairly high dilutions in inhibiting the growth of gram positive cocci and bacilli; it was much less effective against the gram negative members of the coli aerogenes group. Both fumigatin and fumigacin have been found toxic to experimental animals.

Chapter XI

THE ASPERGILLUS NIDULANS GROUP[1]

Outstanding Characters

Conidial heads short columnar usually dark green with primary and secondary sterigmata

Conidiophores smooth walled more or less browned usually sinuate commonly less than 200μ long and terminating in dome like or hemispherical vesicles

Conidia globose echinulate 3 to 4μ in diameter

Perithecia usually present ascospores purple red in color and characterized by equatorial bands

Large thick walled globose bodies termed hülle cells (by Eidam), forming an irregular layer around the perithecia

Aspergillus (Sterigmatocystis) nidulans was described by Eidam in 1883 Since that time the general type of organism covered by his diagnosis has become fairly well known and certain striking characters have become recognized as defining a number of cosmopolitan strains or species commonly referred to as constituting the *Aspergillus nidulans* group

The hulle cells of Eidam first described in *A nidulans* appear also in various transformations in the *A versicolor* *A ustus* and *A flavipes* groups together with other common characters indicative of close relationship They do not appear in the *A clavatus* *A glaucus* or *A fumigatus* groups which are characterized by single sterigmata nor do they occur in the sclerotium forming groups *A candidus* *A niger* *A wentii* *A tamarii* *A flavus* and *A ochraceus*

Group Key

- I Ascospores present
  - A Ascospores smooth walled
    - 1 Equatorial ridges two in number
      - a Ridges 0 5 to 1 0μ wide margin entire
        - Conidial heads green — *A nidulans* (Eidam) Wint
        - Conidial heads white — *A nidulans* mut *alba* Yuill
      - b Ridges 1 5 to 1 8μ wide margin entire — *A nidulans* var *latus* Thom and Raper
      - c Ridges 3 0 to 4 9μ wide margin dissected starlike — *A variecolor* (Berk and Br) Thom and Raper
    - 2 Equatorial ridges usually four in number — *A quadrilineatus* Thom and Raper
  - B Ascospores rough walled — *A rugulosus* Thom and Raper

[1] Abridged from Thom and Raper The *Aspergillus nidulans* group Mycologia Vol XXXI No 6 653–669 Nov–Dec 1939

II Ascospores lacking
A Hülle cells forming irregular masses suggestive of sclerotia
A caespitosus Raper and Thom
B Hülle cells absent, heavy walled sterile hyphae present
A unguis (Emile Weil and Gaudin) Thom and Raper

Aspergillus nidulans (Eidam) Wint in Rab Krypt Fl 1² 62 1884

Synonyms *Sterigmatocystis nidulans* Eidam in Cohn, Beitr Biol Pflanzen 3 392–411 pl 20–22 1883
Diplostephanus nidulans (Eidam) Langeron, Compt Rend Soc Biol Paris, 87 343–345 1922

Colonies upon Czapek's solution agar plane spreading broadly, dark cress green (Ridgway, Pl XXXI) from abundant conidial heads during the first two weeks (Pl IA IVC, and fig 41 A) perithecia developing from the center of the colony outward after the first few days, separately produced often abundant (Pl IVC) sectoring occasional, reverse of colony in varying shades of purplish red during the growing period becoming very dark in age Heads short columnar ranging from 40 to 80µ by 25 to 40µ, commonly 60 to 70µ by 30 to 35µ (Pl IB and fig 4 C), conidiophores commonly sinuous with walls smooth in shades of cinnamon brown (Pl IC) ranging from 60 to 130µ, commonly 75 to 100µ in length about 2 5 to 3µ near the foot increasing to 3 5 to 5µ below the hemispherical vesicle (fig 39 A) vesicle 8 to 10µ in diameter, sterigmata in two series primary 5 to 6µ by 2 to 3µ and secondary 5 to 6µ by 2 to 2 5µ conidia globose, rugulose 3 to 3 5µ in diameter, green in mass

Perithecia developed separately within or upon the conidial layer (Pl IA) globose ranging from 100 to 175µ in diameter commonly 125 to 150µ with outer layer a yellowish to cinnamon colored envelope of scattered hyphae bearing hülle cells up to 25µ in diameter wall composed of one layer of cells dark reddish purple in ripening becoming a mass of 8 spored asci which break down quickly leaving the ascospores free Ascospores purple red lenticular smooth walled with 2 equatorial crests (Pl ID and fig 43 A) spore bodies about 3 8 to 4 5µ in length by 3 5 to 4µ in breadth equatorial crests pleated with margin sinuous and entire ranging from 0 5 to 1µ in width (Table 1)

Diagnosis based primarily upon culture NRRL No 187 (Thom No 4640 5) obtained from the Bainier collection in Paris Other strains assigned to Eidam s species included many isolations from American soil and decaying vegetation as well as cultures from European contributors Common

The range of ascospore measurements found in a representative group of cultures is shown in the accompanying table

In assigning Eidam s species name to the members of this series it is obvious that there are discrepancies He described the perithecium as

Fig 39 Conidial structure in the *Aspergillus nidulans* group × 900 A_1 Typical conidial head of *A. nidulans* NRRL No 187 A_2 Diminutive head of the same strain *B* Typical head of *A caespitosus* NRRL No 1929

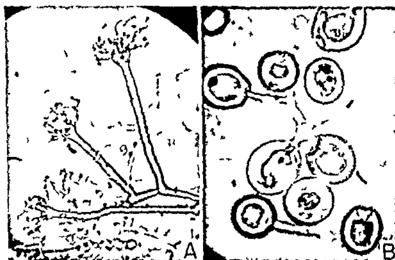
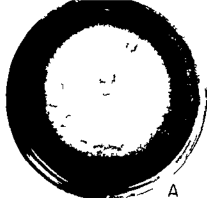
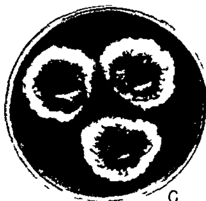
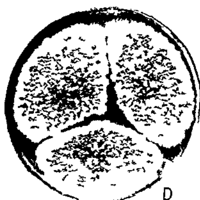


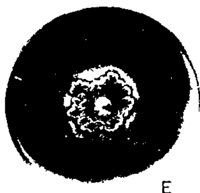
Fig 40 *Aspergillus nidulans* *A* Conidial heads × 370 Conidiophores arise either from the substratum or from aerial hyphae *B* Hülle cells × 740











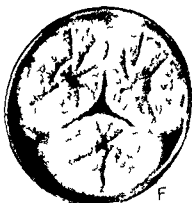


Fig 41 *Aspergillus nidulans* group different species growing upon Czapek's solution agar at room temperature *A* *A nidulans* NRRL No 194 typical strain producing very abundant dark green conidial heads *B* Naturally occurring mutation characterized by white heads isolated from the preceding strain by Edward Yuill and described as *A nidulans* mut *alba* *C* *A rugulosus* NRRL No 207, characterized by heavy perithecium production an almost complete absence of conidial structures and a tendency of colonies to split in central areas as shown *D* *A variecolor* NRRL No 1954 *characterized by the production of* abundant large perithecia *E* *A unguis* NRRL No 216 characterized by an absence of perithecia and hülle cells *F* *A caespitosus* NRRL No 1929 characterized by the production of masses of hülle cells suggesting abortive perithecia

having a firm almost sclerotioid wall whereas the wall is found to contain but one layer of cells He figured the asci as few and scattered in a mycelial matrix within which ascospore production occupied many weeks One isolated strain in our collection produces asci in this manner For it the varietal name *A nidulans* var *latus* was proposed by Thom and Raper (1939) on account of very broad crests on the ascospore in contrast to the usual types in *A nidulans* which come much more closely to those indicated in Eidam s figures

TABLE I

Ascospore variation in strains of Aspergillus nidulans

Culture number	Over-all dimension of spores	Width of crests	Dimensions of spore bodies
NRRL 193	6.2–6.6 x 3.6–3.8µ	1.0µ ±	4.2–4.6 x 3.6–3.8µ
NRRL 188	6.0–6.6 x 3.6–3.9µ	1.0µ ±	4.0–4.6 x 3.6–3.9µ
NRRL 189	6.0–6.4 x 3.6–3.8µ	1.0µ ±	4.0–4.4 x 3.6–3.8µ
NRRL 194	5.4–5.8 x 3.6–3.8µ	0.6–0.8µ	4.0–4.4 x 3.6–3.8µ
NRRL 195	5.4–5.8 x 3.6–3.8µ	0.6–0.8µ	4.0–4.4 x 3.6–3.8µ
NRRL 191	5.4–5.8 x 3.6–3.9µ	0.6–0.8µ	4.0–4.4 x 3.6–3.9µ
NRRL 187	5.4–5.8 x 3.6–3.8µ	0.7–0.8µ	3.9–4.3 x 3.6–3.8µ
NRRL 192	5.0–5.4 x 3.6–3.8µ	0.5–0.6µ	4.0–4.4 x 3.6–3.8µ
NRRL 198	4.8–5.4 x 3.6–3.8µ	0.5–0.6µ	3.8–4.4 x 3.6–3.8µ
NRRL 199	4.8–5.2 x 3.6–3.8µ	0.5µ ±	3.8–4.2 x 3.6–3.8µ

White mutant from culture NRRL No 194 described by Yuill as *Aspergillus nidulans* mut *alba*

A nidulans mut *alba* Yuill in Jour of Botany (London) 175 pl 618 1939

Colonies of the variety on Czapek s solution agar differ from the species in the entire absence of green color (fig 41 B) The ascospores have the characters of the species

Culture obtained by Yuill as a mutant from a normal green strain of *A nidulans* under investigation in his laboratory Our record number is NRRL 195 the normal and parent strain is NRRL 194

Aspergillus nidulans var *latus* Thom and Raper Mycologia 31 657 1939

Colonies on Czapek s solution agar differing from the species in colony development characterized by a felt of predominantly sterile mycelium few conidial heads fairly abundant perithecia developed in the mycelial felt and each surrounded by a thick covering of hulle cells (fig 42 C) very slowly ripening and containing few and scattered asci in abundant sterile mycelium Ascospore bodies smooth walled purple red 3.8 to 4.5µ by 3.5 to 4µ with crests 1.5 to 1.8µ in width

Type culture NRRL No 200 received from the Centraalbureau in 1909 and remaining constant in culture since that time Its antecedent history is not known

Aspergillus quadrilineatus Thom and Raper Mycologia 31 660, figs 2-4 1939

Colonies on Czapek's solution agar spreading plane or slightly wrinkled with tendency toward floccosity central area gray with a definite purplish tinge, and olive green conidial areas toward the margin occasionally as sectors, perithecia developing separately but abundantly throughout the colony, reverse purplish red, heads short columnar, green, mostly 60 to 70μ by 30 to 35μ occasionally larger or smaller, conidiophores sinuate smooth walled dull brownish in color 50 to 75μ in length by 3 5 to 4 5μ wide, broadening to 7 5 to 9μ at the hemispherical vesicular areas primary sterigmata 5 to 6μ by 2 to 3μ, secondary sterigmata 5 to 7μ by 2 to 2 5μ conidia globose, pale yellow green rugulose, 3 to 4μ in diameter perithecia enveloped by hulle cells, light brownish in color spherical partially embedded in the mycelial felt (fig 42 D) about 125 to 150μ in diameter including the enveloping hülle cell layer, with perithecial wall 1-cell layer in thickness, ripening quickly and with ripe asci breaking down to leave the ascopores free ascospores purple red, lenticular with smooth wall, with spore body 4 to 4 8μ by 3 4 to 3 8μ, and with two pleated equatorial crests about 0 5μ in width paralleled by a secondary narrower pair (fig 43 B) which are sometimes indistinct

Type NRRL No 201 (Thom No 4138 N8) from New Jersey soil and kept in culture since 1916 Other strains examined include isolations from Texas Colorado Louisiana, and Maryland

Aspergillus rugulosus Thom and Raper, Mycologia 31 660-663, fig 4 1939

Colonies on Czapek s solution agar slowly and restrictedly growing (fig 41 C) buckled or wrinkled in a mass 2 to 3 mm deep enveloping abundant perithecia at different depths often eventually splitting in the central area purple gray to purple brown in age with green heads sparsely produced and hence not generally evident occasionally seen as small groups and marginal extensions into drying media reverse in shades of deep purple red conidial heads short columnar 75 to 100μ by 30 to 40μ conidiophores *sinuous smooth walled pale brownish in color* 50 to 80μ long slender, varying up to 5μ in width, then enlarging to vesicular hemispheres 8 to 10μ in diameter primary sterigmata 7 to 8μ by 3 to 3 5μ secondary sterigmata 6 to 7μ by 2 5 to 3μ conidia globose green rugulose 3 to 4μ

Perithecia very abundant often imbedded in the mycelium as 2 or 3 layers and each surrounded by hyphae and dark brown hulle cells (fig 42 E) globose 225 to 350μ in diameter including mycelial coverings with dark reddish purple walls of one cell thickness quickly ripening and breaking down to leave ascospores free asci 10 to 11μ in long axis asco pores purple red lenticular walls conspicuously rugulose (fig 43 C) with spore bodies 4

to 4 4μ by 3 6 to 3 8μ and with 2 pleated equatorial crests with sinuate and entire margins about 0 5 to 0 6μ in width

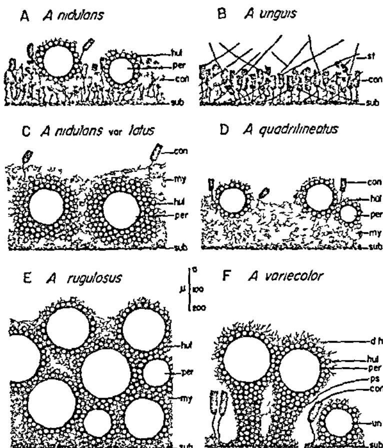


Fig 42 Diagrammatic representations of cross sections of colonies of different species of the *Aspergillus nidulans* group showing the relative abundance of conidial heads and perithecia and the manner in which these structures are borne *con* conidial heads *d h* mantle of divergent hyphae *hul* hülle cells *my* mycelial felt *per* perithecia *ps* pseudostalk of hülle cells and sterile hyphae *st* long thick walled sterile hyphae *sub* substratum and *un* unstalked perithecium Scale approximate (Reprinted from Thom and Raper "The *Aspergillus nidulans* Group Mycologia 31 653-669 1939)

Cultures studied included Type NRRL No 206 (Thom No 4138 T11) from New Jersey soil as discussed by Thom and Church in *The Aspergilli* p 138 and also isolates from Washington D C Texas Nebraska and California Very common in soil

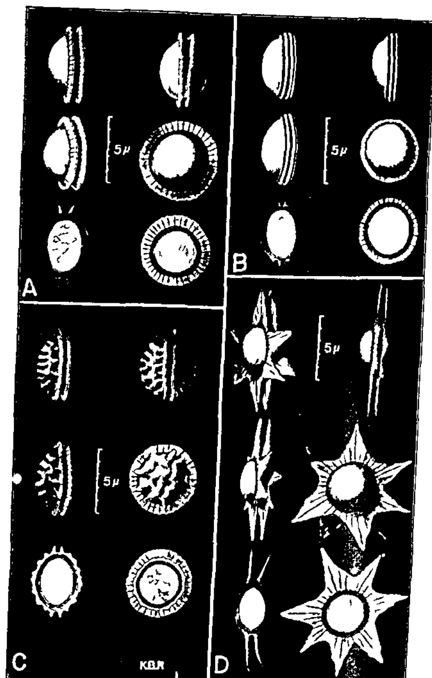


FIG 43 Ascospores of different species of the *Aspergillus nidulans* group *A A nidulans* *B A quadrilineatus* *C A rugulosus* *D A variecolor* In each species upper left and right and center left spores represent surface profile views center right surface in face view lower left optical section in profile and lower right optical section in face view (Reprinted from Thom and Raper The *Aspergillus nidulans* Group Mycologia 31 653–669 1939)

Culturally and microscopically the above strains present similar pictures with the exception of the strain recently received from Bliss in California (NRRL No 211) In contrast to the others this culture produces abundant conidial heads and relatively fewer perithecia The ascospores and conidial structures however duplicate those of the typical strains hence we do not at present feel warranted in designating this as a variety, or otherwise separating it from the species *A rugulosus*

Aspergillus variecolor (Berk and Br) Thom and Raper in Mycologia 31 663–667 fig 4D and fig 5 1939

Synonyms *Emericella variecolor* Berk and Br in Berkeley, Introd Crypt Bot p 340–341 fig 76 1857 See Patouillard Bull Soc Myc Fr 7 43–49 pl 4 fig 6–12 1891
Inzengaea erythrospora Borzi Jahrb Wiss Bot (Pringsheim) 16 450–463 pl 19, 20 (1884) 1885
Emericella medias Chowdhury and Mathur Ann Myc 36 61–63 1938
Aspergillus stellatus Curzi Rend Acad Naz Lincei 19 424–428 fig 1 1934

Colonies on Czapek s solution agar with vegetative mycelium largely submerged sparse spreading slowly in the agar, producing green heads freely in the center of the colony, less abundantly in the outer areas large gray perithecia produced in clusters in colony center and at the margin in some strains, with smaller perithecia scattered through the intervening thinner areas of the colony (fig 44 A) in other strains producing large perithecia abundantly throughout the colony (Pl IVD) reverse color in shades of purple red Conidial heads green columnar (fig 44 D) relatively long mostly 100 to 200µ occasionally up to 300µ by 30 to 40µ conidiophores arising directly from submerged hyphae straight with smooth walls cinnamon brown in color mostly 140 to 200µ long by 3 to 5µ in diameter broadening gradually to become hemispherical vesicles about 8 to 10µ in diameter primary sterigmata 7 to 8µ by 3 to 4µ secondary sterigmata 8 to 9µ by 2 5 to 3µ conidia globose rugulose 3 to 3 5µ perithecia when clustered (fig 44 Aa) 300 to 400µ in diameter surrounded by a felt of hyphae and hülle cells and supported by masses of hyphae and hülle cells forming false stalks (fig 42 F) giving the structures a pyriform appearance (fig 44 B) scattered perithecia much smaller (fig 44 C) and with envelope of supporting cells often much reduced in mass hülle cells abundant and essentially like those of the species *A nidulans*

Perithecial wall when stripped of enveloping cells purple red brittle composed of a single layer of cells asci quickly ripening and breaking down to leave the cavity filled with ascospores ascospores purple red with spore bodies lenticular and 3 6 to 4µ by 2 8 to 3µ with two prominent equatorial



FIG 44 *Aspergillus variecolor* A Central area of colony a cluster of large pseudostalked perithecia b scattered smaller unstalked perithecia (see also figure 42 F) c scattered conidial heads × 6 B Enlarged view of large pseudostalked perithecium × 65 C Enlarged view of small unstalked perithecia × 65 D Enlarged view of conidial heads × 65 (Reprinted from Thom and Raper The *Aspergillus nidulans* Group Mycologia 31 653-669 1939)

crests, up to 3 5µ in width, pleated and cut to give a stellate appearance to the ascospores (fig 43 D)

The above description is based primarily upon a culture received from Prof Verona in Italy and carried in our collection as NRRL No 212 (Thom No 5602 3)

Bliss forwarded a culture (NRRL No 214) isolated from date fruits in California which differs from the above in the following particulars (1) Conidial heads are produced abundantly (2) the mycelium is not predominantly submerged and (3) the colonies in reverse are deep purple However the ascospores of the two strains are strikingly similar in size and pattern the perithecia of each appear pyriform in shape, and the conidial structures of the two are essentially alike More recently a strain has been isolated from Arizona soil (NRRL No 1954) which produces abundant large stalked perithecia but very few small perithecia or conidial heads There is thus evidence of considerable natural variation among members of this species All of these strains have the same type of stellate ascospore

Since the type of ascospore described here had already been assigned to *Emericella* and *Inzengaea* consideration of the literature of these genera is necessary

Emericella variecolor genus and species new was described by Berkeley and Broome in 1857 as doubtfully a Gasteromycete or possibly a lichen The perithecium with a mass of stellate spores was considered as gastromycetous in character while what we now recognize as the hulle cells of Eidam (figured) suggested to them the possibility of an algal associate Berkeley's material was also examined by Montagne and part of it deposited in the Museum d Histoire Naturelle de Paris This was reexamined by Patouillard in 1891 and its ascomycetous nature determined No conidial apparatus was found by either Berkeley or Patouillard

Inzengaea erythrospora as the type species of a new ascomycetous genus was figured and described by Borzi in 1885 showing stellate red ascospores and the hülle cells of Eidam Borzi s figure showed a coremium like conidial apparatus which was designated *Coremium Borzianum* by Saccardo Ed Fisher in 1893 transferred the species to *Emericella* of Berkeley and placed the genus next to *Aspergillus* in the *Pflanzenfamilien* Saccardo (in Syll 9 610) on the other hand accepted *Inzengaea* and dropped *Emericella* because of the errors in description and placement by Berkeley Borzi figured the spores of *A variecolor* (*Inzengaea erythrospora*) correctly but obviously misinterpreted the germination of the ascospores since he showed them splitting as if turned 90 bringing the crest perpendicular to the center of the valve instead of attached to its edges

There the taxonomic situation stood until Vuillemin in 1927 concluded that the ascosporic apparatus the stellate red ascospores and the cells of Eidam as clearly shown in their figures and material showed the identity

of *Emericella* and *A nidulans* He therefore transferred *A nidulans* to *Emericella* as the oldest established genus and apparently did not even consider *Inzengaea*

Ciferri (1938) has recently completed a study of *Emericella variecolor* embracing cultural investigations together with a review of the literature of the genus He did not recognize the close relationship of this fungus to *Aspergillus nidulans* and was apparently unmindful of the likeness of their conidial structures and the essential similarity of their perithecia and ascospores

In 1934 Curzi described as *Aspergillus stellatus* a fungus characterized by hulle cells and red stellate ascospores However, he apparently did not know of either Berkeley's or Borzi s earlier designation of a similar fungus It is unfortunate that his exceedingly descriptive binomial must be reduced to synonymy

Fortunately for this discussion, cultures NRRL Nos 212 214 and 1954 present both the stellate spores and apparently stalked perithecia figured by Berkeley, Patouillard, and Borzi (Compare figures 42 F and 44, B with Berkeley s figure 76a Patouillard's figures 7 and 8 plate 4 and Borzi's figure 10 plate 19) This made possible a restudy of the whole morphologic situation from fresh material The perithecial body itself was found not to be stalked but to rest upon a sterile mass of hülle cells and mycelium giving the superficial appearance noted by earlier observers

The coremium of Borzi remains unaccounted for Obviously in Borzi s discussion the material was rotten olives and very old No cultures were made The conidia producing apparatus (figured) differs essentially in type from the conidial apparatus of the Aspergillaceae with which Fischer correctly placed *Emericella* because of its perithecia and ascospores We are convinced that the coremia belonged to some other fungus

It is not possible to separate the perithecium of this fungus from that of the other species in the *A nidulans* group nor are there characters to take this type of perithecium out of a genus with the yellow perithecium of the great *A glaucus* series of species which are widely known Consistent with the policy of keeping the Aspergilli in one group both *Emericella* and *Inzengaea* are dropped for purposes of this discussion

Aspergillus caespitosus Raper and Thom in Mycologia 36 563-565 fig 4 1944

Colonies varying markedly upon different media, upon Czapek s solution agar rather slow growing attaining a diameter of 6 to 8 cm in three weeks at room temperature plane or somewhat furrowed mycelium largely submerged and extremely tough tearing with difficulty producing numerous dark green hemispherical to loosely columnar heads in central colony areas

characterized particularly by clusters of irregular ovoid to elliptical thick walled hülle cells at first colorless becoming reddish purple in age scattered

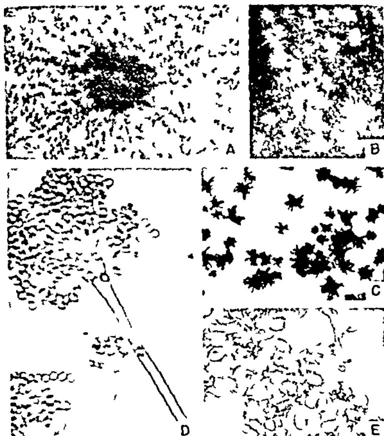


FIG 45 *Aspergillus caespitosus* NRRL No 1929 *A* Portion of colony on Czapek s solution with 1 percent liver extract, showing crowded conidial heads in central portion and scattered hülle cell masses in surrounding areas two weeks old incubation at room temperature × 1.8 *B* Portion of colony enlarged showing hülle cell masses × 10 *C* Silhouettes of conidial heads developed on hay infusion agar × 48 *D* Typical conidial head showing form of vesicle and arrangement of sterigmata × 600 *E* Portion of hülle cell mass showing irregular size and form of component elements × 265 (Reprinted from Raper and Thom New Species of Aspergilli from Soil Mycologia 36 Nov-Dec 1944)

unevenly (fig 45 A and B) or arranged in irregular concentric zones reverse colorless at first becoming dark reddish purple in age particularly beneath the hülle masses odor none Conidial heads dark dull yellow green to

empire green (Ridgway, Pl XXXII) generally hemispherical to loosely columnar, mostly 75 to 125μ in diameter Conidiophores straight or slightly sinuous (fig 45 D), mostly 250 to 325μ in length, occasionally up to 350μ by 5 0 to 6 5μ in diameter of approximately uniform diameter throughout relatively thick walled (1 2 to 1 5μ in basal portion to 0 8 to 1 0μ in terminal area), smooth, tan to light brown in color Vesicle slightly elongate, the upper hemisphere loosely covered by sterigmata (fig 45 D) the lower half sterile and often lightly colored, mostly 15 to 20μ in diameter Sterigmata in two series (fig 45 D) primaries normally 6 5 to 8 5μ by 3 5 to 5 0μ, secondaries 6 5 to 8 0μ by 3 0 to 4 5μ typically bottle form but commonly much swollen and often quite irregular in form and dimensions Conidia globose, spinulose, green, mostly 3 5 to 4 5μ rarely larger, hulle cells very abundant thick walled, irregularly globose ovoid or elliptical (fig 45 E) ranging from 12 to 18μ in globose cells to 12 to 15μ by 25 to 30μ in the most elongate bodies forming compacted masses of indefinite size extremely tough and in age becoming almost sclerotioid at first colorless but in age characterized by an abundant reddish purple intercellular pigmentation

Colonies upon malt agar characterized by a dense stand of erect conidiophores bearing hemispherical to radiate or loosely columnar heads of dark green color approximately empire green (Ridgway, Pl XXXII) and the complete absence of hulle cells, reverse in light brown shades odor none Details of morphology as upon Czapek's solution agar

Colonies upon hay infusion agar like those upon malt except less heavily sporing

Strains include NRRL No 1929 (type) isolated from Arkansas soil and other isolations from Arizona and Texas soils

This species is of particular interest because of its apparent transitional position between the *A nidulans* group and *A ustus* In the character of its conidiophores its reddish purple pigmentation, and in the general color and markings of its conidia it retains the characters of *A nidulans* and closely related species In the absence of fertile perithecia and ascospores the predominantly hemispherical shape of its conidial heads, and in the variable and irregular form of its hulle cells it is strongly suggestive of the *A ustus* series While we are convinced of its intermediate position between the *A nidulans* group and *A ustus* we place it with the former since we believe it is most closely allied to this group It is believed significant that superficially cultures of *Aspergillus caespitosus* and *Aspergillus variecolor* (Berk and Br) Thom and Raper (1939) are strikingly similar upon Czapek s solution agar This similarity is particularly marked when plates are viewed in reverse since an intense pigmentation marks the under surface of perithecia in the latter case and the under surface of older hülle masses in the former

Aspergillus unguis (Emile Weil and Gaudin) Emend Thom and Raper, Myc 31 p 667 fig 6 1939

Synonyms *Sterigmatocystis unguis* Emile Weil and Gaudin Arch Med Expt Anat Path Paris 28 463–465 fig 4 1919
A koakiashanensis Shih Lingnan Sci Jour 15 (3) 369 p 16 fig 2 1936

Colonies on Czapek s solution agar restrictedly growing plane spreading at the margin as irregular lobes (fig 41 E) yellowish green green to dark green becoming brown in age, without perithecia or hülle cells Mycelial preparations show striking sterile thick walled hyphae with walls in brown



Fig 46 Sterile spicule hyphae of *Aspergillus unguis* *A*, Cluster of sterile hyphae × 3 0 *B* Apex of sterile hypha × 740 *C* and *D* Mid portions of sterile hyphae showing thick rough ned walls × 740 (Reprinted from Thom and Raper "The *Aspergillus nidulans* Group Mycologia 31 653–663 1939)

shades irregularly roughened (fig 46) tapering to a blunt point arising sometimes from foot-cells suggesting the origin of conidiophores sometimes apparently from mycelial cells often up to 1 000μ or more in length slant ing upward but usually ri ing only slightly above the conidial area (fig 42 B)

Conidial heads columnar 75 to 150μ by 40 to 50μ conidiophores smooth walled dull brown in color mostly 45 to 65μ in length by 3 to 5μ in diameter enlarging to ve icular hemispheres 9 to 12μ in diameter primary sterigmata 5 to 6μ by 2 5 to 3μ econdary sterigmata 5 to 6μ by 2 to 2 5μ conidia globose rugulose, dull green 2 5 to 3 5μ in diameter

Cultures of *A unguis* are obtained frequently from medical laboratoies apparently as more or less active pathogens but occa ionally isolated from

soil and decaying organic matter The question whether the non ascosporic members of the group have merely dropped the ascogenous phase or constitute a separate species was answered when more complete examination showed the sterile or spicule hyphae to be regularly produced in the non ascosporic but never found in ascosporic series

Pathogenicity

Aspergillus nidulans in some of its forms and variants has been demonstrated as a parasite in human nails (onychomycosis), often enough to establish its pathogenicity *A nantae* Pinoy (1927) probably belongs here although the data are mainly pathological, hence not adequate for definite identification of the organism *A nidulans* forme *cesarii* Pinoy (1915) isolated from a mycetoma of the lung of a donkey, and *A nidulans* var *nicollei* Pinoy (1906) isolated in Tunis from a case of mycetoma, or madura foot, represent additional strains which were at least secondary pathogens The nearly related *A unguis* is usually the more common form isolated from human material *A Brodeni* (Mattlet) Dodge (1935) from a bronchomycosis in Africa might have been close to *A unguis* *A nidulans* and *A unguis* are both widely distributed as saprophytes hence are constantly encountered as components of dirt reaching the extremities by contamination Infection of the air passages is comparatively rare

Occurrence and Economic Importance

In addition to their role as occasional disease producing agents members of the *A nidulans* group are believed to be significant in decomposition processes They are among the molds most commonly isolated from soil, and very frequently appear in considerable abundance upon vegetable material undergoing slow decomposition *Aspergillus rugulosus* *A quadrilineatus* and *A caespitosus* occur most frequently in soils from the comparatively dry warm soil of Texas Arizona and adjoining areas *Aspergillus variecolor* has been isolated from olives in Italy date fruit in California and from Arizona soil *Aspergillus nidulans* is abundant and cosmopolitan in its distribution

CHAPTER XII

THE ASPERGILLUS USTUS GROUP

Outstanding Characters

Colonies more or less floccose at first white but becoming dull in age in most members varying from olive gray through reddish brown to fuscous as conidial structures develop
Conidiophores in yellow brown shades smooth
Heads irregular in form ranging from more or less radiate to hemispherical to loosely columnar
Vesicles hemispherical sterigmata in two series loosely arranged
Conidia roughened 3 0 to 5 0μ varying from echinulate to marked with conspicuous color bars and ranging in color from pale blue green through olive green shades to deep brown (fuligineus)
Hulle cells regularly present thick walled elongate often more or less curved and twisted

Included here are representatives of a most abundant and widespread group of fungi especially common in soil and upon decaying vegetation

Group Key

Colonies predominantly floccose heavy sporing hülle cells not aggregated in small clusters *A ustus* (Bain) Thom and Church
Colonies more or less floccose light sporing hülle cells aggregated in small clusters *A granulosus* Raper and Thom

Aspergillus ustus (Bainier) Thom and Church in The Aspergilli p 152 1926

Synonym *Sterigmatocystis usta* Bainier in Bul Soc Bot France 28 78 1881

Colonies upon Czapek s solution agar spreading broadly plane sulcate or umbonate rarely zonate more or less felted or floccose, at first white becoming olive gray, yellow brown fuscous or russet to purplish vinaceous with the development of mature conidial structures (Pl IV E, and figs 48 A and B) generally heavy sporing with some conidiophores arising from the substratum but more abundantly from aerial hyphae reverse in shades of yellow orange, and brown to almost black in age odor not pronounced Heads radiate to irregularly hemispherical sometimes loosely columnar commonly splitting into more or less well-defined columns in age very variable in size ranging in color from dull green or olive gray through gray

ish brown to fuscous or fuligineus Conidiophores arising from submerged hyphae (fig 47 A) ranging up to 500μ long by 3 to 6μ aerially borne conidiophores, ranging from very short (fig 47 A_1) up to 125μ by 2 to 5μ sinuous sparsely septate, with walls rather thin, smooth, and uniformly colored some shade of brown Vesicles hemispherical to subglobose 8 to 20μ in diameter, smaller in some strains (fig 47 A) Sterigmata colorless or

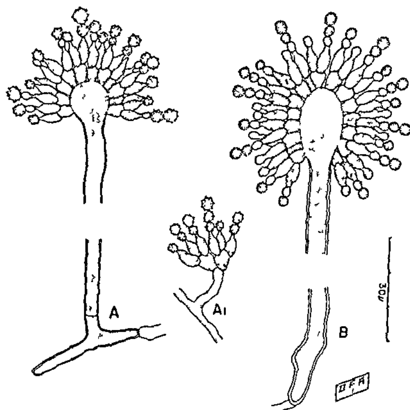


FIG 47 *Aspergillus ustus* group × 840 A Typical conidial head showing comparatively loose sterigmata in two series and conspicuously roughened conidia strain NRRL No 278 A_1 Diminutive head as often seen in strain NRRL No 275 *B* Typical head of *Aspergillus granulosus* NRRL No 1932

colored semi radiate loosely arranged into two series primary sterigmata 4 to 7μ by 3μ secondary sterigmata 5 to 7μ by 2 0 to 2 5μ Conidia globose 3 5 to 5 0μ roughened echinulate to marked with conspicuous color bars ranging from greenish through olive gray to yellow brown or fuligineus Many strains producing thick walled hülle cells (fig 49 E) ranging in form from irregularly ovate or elongate in some strains to serpentine helicoid or twisted in others essentially as in *Aspergillus flavipes*

Upon malt agar, colonies frequently heavier sporing and conidial heads generally tending to run to dull gray green shades rather than brown Very common in soil and decaying vegetation Species diagnosis represents a composite based upon many isolations from this country and abroad

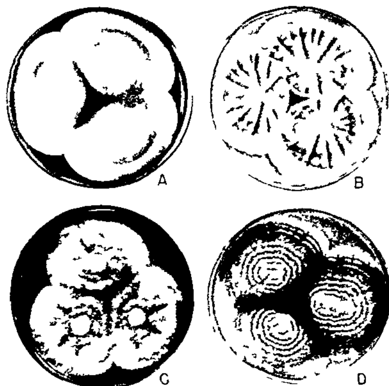


FIG 48 *Aspergillus ustus* cultures growing upon Czapek s solution agar at room temperature 10 days *A* Strain NRRL No 275 characterized by loose floccose colonies and moderate sporulation *B* Strain NRRL No 278 characterized by heavier spore production and the presence of abundant hülle cells *C A ustus* var *laevis* NRRL No 1852 characterized by loose floccose colonies and conidial heads often near brick red in color *D* Strain NRRL No 1974 characterized by the production of very abundant hülle cells in concentric zones

Individual strains differ markedly in their general habit and colony coloration in the color of their fruiting structures in the marking and coloration of their conidia in the presence or absence of hulle cells and in the form of these structures when present By a deliberate selection of strains one can find sufficient difference to warrant the assignment of specific designa

tions to particular cultures Yet all possess the characters noted above and so constitute a well-defined group whose variations are matters of detail Such differences as occur, *moreover, tend to become bridged as comparative* cultural and microscopic studies of many isolations are made, hence the use of these differences becomes of questionable value for diagnostic purpose We have considered it desirable, therefore, to include the whole series under one name as a single species aggregate *Aspergillus ustus* and to call attention to some of the major differences which one may expect to encounter among the members of this species Although Bainier was not sufficiently *explicit in his description of Sterigmatocystis usta to* enable us to identify with certainty the form with which he worked his usage is accepted upon (1) the basis of priority, and (2) the receipt of a culture (Thom No 4640 488) from his laboratory labeled *Sterigmatocystis usta* which possessed the basic characters of the group as herewith set forth

Long after the publication of *Sterigmatocystis usta*, Bainier described a *second species belonging to this group, Sterigmatocystis insueta (Bul Soc* Mycol *France 24 85–87, Pl VIII Fig 1–13 1908) and emphasized* the fuligineus character of its colonies, the predominant origin of brown fruiting structures from aerial mycelium, the larger size and the darker color of its conidia which were characterized by the presence of pronounced color bars Strains showing these characters probably represent the type most commonly encountered among miscellaneous isolations of forms belonging to this group Recognition of these forms as constituting a distinct *species has been considered, but in the absence of any clearly* definable line of separation from *A ustus* it is believed desirable to leave them within the somewhat extended framework of this species Cultures possessing these characteristics very commonly exhibit hülle cells varying in different strains from ovoid to irregularly elongate to serpentine, helicoid or otherwise twisted *One strain showing much twisted hülle cells* was isolated and contributed by Thaxter under the manuscript name *A helicophorus* Typically the conidiophores of these forms are grayish brown commonly quite dark The vesicle and sterigmata are likewise frequently colored Conidial color as seen under high magnifications varies with age from pale to olive green to fuligineus, and conidial markings from fairly coarse echinulations to intensively colored bars and tubercles

Possibly unaware *of the existence of Sterigmatocystis usta* and *S insueta* (since no mention is made of either species) Abbott (1926) subsequently described *Aspergillus minutus* His description indicated that he was dealing with a strain essentially similar to those considered by Bainier as *S insueta* This is confirmed by examination of his type culture NRRL No 283 (Thom *No 4894 2) A second culture* NRRL No 285 (Thom No 4894 1) received from Abbott under the manuscript name *A humus* likewise represents a member of this series and differs from the more

common forms only in its more floccose habits and in the production of somewhat smaller conidial heads

Among the more striking members of the *Aspergillus ustus* series examined are two isolations from soil collected in Panama and Mexico respectively, which upon Czapek's solution agar normally produce strongly zonate colonies consisting of alternating areas of crowded conidial heads and heavy hülle cell development (fig 48 D) Except for this difference in colony appearance, however these strains appear to be typical of the species as described above

A single strain NRRL No 1852 isolated from Louisiana soil possesses conidial heads of a dull brick red color (Ridgway Pl XXXIX russet vinaceous to sorghum brown) and abundant much twisted hülle cells While Blochwitz s description is too inadequate to permit of detailed comparison it is suggested that this strain may represent his *Aspergillus ustus* var *laevis* (Ann Mycol 32(1/2) 4 1934) which was described as characterized by red conidia and crooked hülle cells In strain NRRL No 1852 the conidia are conspicuously reddish en masse but appear only slightly colored when viewed with high magnifications In contrast to most strains of the *Aspergillus ustus* group the conidia are finely echinulate rather than coarsely roughened It is suggested that this form (fig 48 C) may represent a transition in the direction of *Aspergillus flavipes* since the latter species is likewise characterized by much twisted hülle cells brown conidiophore walls and conidia which may show a reddish color in some strains but are smooth in all

Aspergillus granulosus Raper and Thom in Mycologia 36 565–568 fig 4 1944

Colonies upon Czapek s solution agar growing well attaining a diameter of 8 to 10 cm in two to three weeks at room temperature plane or irregularly furrowed predominantly floccose uneven in texture buff to dull brown in color from felted sterile mycelia conidial heads few in number and generally arising from the substratum direct less often from aerial hyphae commonly appearing in clusters pale blue green in color colonies characterized particularly by abundant small colorless clusters of irregularly globose ovoid or elliptical thick walled hülle cells which superficially suggest perithecial initials and which in mass give to the colony a semi granular appearance (fig 50 B E and F) reverse in shades of dull yellow and brown slight mushroom odor Conidial heads few in number commonly clustered in small groups most abundant at colony margin sometimes occurring on tufts of aerial hyphae hemispherical to radiate 75 to 125µ in diameter very loose consisting of comparatively few divergent spore chains (fig 50 C) approximately pale niagara green in color (Ridgway Pl XXXIII) Conidiophores erect straight nonseptate mostly 350 to

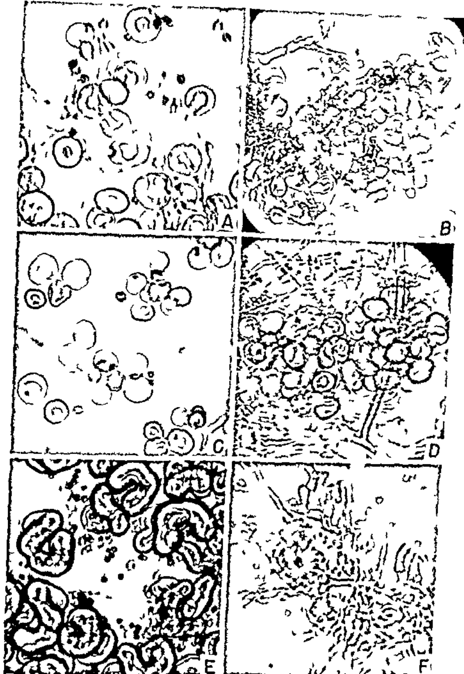


FIG 49 Hülle cells *A* Characteristic thick walled globose to subglobose hülle cells of the *Aspergillus nidulans* group *A variecolor* NRRL No 1951, × 450 *B*, *Aspergillus caespitosus* strain NRRL No 1930 hülle cells irregular in form and often poorly developed but of the same general pattern as *A nidulans* × 2 5 *C* *Aspergillus janus* NRRL No 1 67 hülle cells approximately globose and of the same basic type as in *A nidulans* × 450 *D* *Aspergillus granulosus* NRRL No 1932 hülle cells globose to ovoid somewhat intermediate between *A nidulans* and *A ustus* × 275 *E* *Aspergillus ustus* NRRL No 280 characteristic hülle cells elongate and much twisted × 450 Hülle cells in *Aspergillus flavipes* are of the same pattern *F* *Aspergillus carneus* NRRL No 1976 heavy walled strongly septate mycelium suggestive of hülle cells × 275

500μ in length 5 5 to 8 0μ in diameter approximately uniform in width throughout thin walled smooth tan to light brown in color often slightly

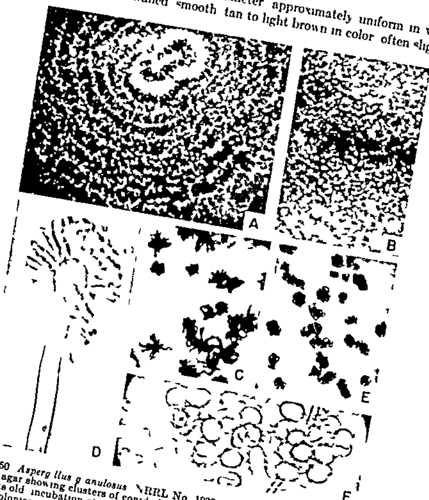


Fig 50 *Aspergillus granulosus* NRRL No 1932 *A* Portion of colony on hay infusion agar showing clusters of conidial structures and small masses of hulle cells two weeks old incubation at room temperature × 1 5 *B* Portion of marginal area of two colonies on malt extract agar showing characteristic granular appearance resulting from numerous small clusters of hülle cells × 1 5 *C* Silhouettes of conidial heads × 45 *D* Conidial head showing slightly elongate vesicle sterigmata in two series and constriction in conidiophore just beneath the vesicle × 750 *E* Small clusters or granules of hulle cells × 48 *F* Double cluster of hülle cells much enlarged × 265 (Reprinted from Raper and Thom New Species of Aspergilli from Soil Mycologia 36 Nov -Dec 1944)

constricted just beneath the vesicle (figs 47 B and 50 D) Vesicle ovate to elliptical thin walled and easily broken largely covered by sterigmata 12 to 18μ in diameter by 15 to 25μ in length Sterigmata in two series

both comparatively short and stout primaries 3 5 to 5 0μ by 3 0 to 4 0μ, secondaries 4 0 to 5 5μ by 3 0 to 3 5μ commonly bottle form Conidia globose, pale green delicately echinulate *Mostly 4 8 to 5 5μ in diameter*, rarely larger Hulle cells abundant, irregularly globose ovoid or somewhat elongate, commonly 12 to 30μ in long axis walls heavy, 4 to 5μ in thickness *borne primarily in small colorless clusters* which are quite conspicuous at colony margins and lend to them a characteristic granular appearance

Colonies upon malt extract agar showing an accentuation of hülle cell development (fig 50 B) and a reduction in conidial heads Otherwise duplicating the cultural picture presented upon Czapek's solution agar

Colonies upon hay infusion agar (fig 50 A) thin but broadly spreading characterized by scattered clusters of hulle cells and erect conidial fructifications giving to the culture a sparsely granular appearance

Type culture NRRL No *1932* was *isolated in 1942 from a sample of soil* collected in Fayetteville Arkansas, and contributed by Mr F R Earle *Additional strains* have been isolated from soils collected in Texas Arizona and Costa Rica *It is believed common in soils where the temperature* remains at a high level during part or all of the year

Different strains vary materially in the number of conidial heads produced upon common laboratory media such as Czapek s solution and malt extract agars ranging from abundant heads in some to only widely scattered heads in others All fruit reasonably well however upon hay infusion agar, the medium upon which original isolations were made

The brown color of the conidiophores, the presence of ovoid to somewhat irregular hulle cells, and the green color of its conidia place this species in the group with *A ustus* It differs markedly from the more common representatives of this group however, in the lighter and persistently green *color of its conidia the small clusters rather than irregular masses* of hulle cells and in possessing somewhat more elongate vesicles In this latter character it suggests *Aspergillus flavipes* but is in turn excluded from this group by the green color of its spores

Occurrence and Economic Importance

Representatives of the *Aspergillus ustus* group are perhaps *the most* abundant of all aspergilli in soil They regularly occur in large numbers and in considerable variety The common species *A ustus*, occurs alike in cultivated and forest soils and in approximately equal abundance in soils from southern and from north temperate areas *A granulosus* on the contrary, has been isolated only from southern sources Members of the group are not known to be particularly active agents of decomposition but their great abundance in nature is believed indicative of a significant role in many decay processes

Their biochemical activities and potentialities are almost completely unknown

Chapter XIII

THE ASPERGILLUS FLAVIPES GROUP

Outstanding Characters

Conidiophores smooth in some shade of yellow with color often confined to the outer layer

Heads barrel form to columnar when well developed white or slowly becoming some shade of vinaceous buff to avellaneous (Ridgway Pl XL)

Vesicles subglobose to elliptical

Conidia colorless smooth thin walled

Hülle cells generally present helicoid or variously twisted

The range of variation presented within the group has led workers with only a few representatives before them to offer names and descriptions for the strains under observation However when large numbers of strains are brought together and cultivated upon a considerable range of substrata the continuity of the group as a natural and related series of strains becomes apparent Further study of this whole group may lead to separation upon lines accounting for certain published descriptions not at present identifiable For purposes of the present manual however it is believed desirable to broaden the description of *Aspergillus flavipes* (Bain and Sart) Thom and Church sufficiently to include a closely related series of organisms rather than restrict it to the particular strains studied by Bainier

Aspergillus flavipes (Bain and Sart) Thom and Church The Aspergilli p 155 1926

Synonym *S flavipes* Bainier and Sartory (Bul Soc Myc France 27 90–96 Pl III fig 1–6 1911)

Colonies upon Czapek s solution agar rather slow growing becoming 3 to 5 cm in diameter in about 10 days mycelium yellowish dull buff commonly becoming brownish in age heads pale to dull buff in some strains to avellaneous or even very light cinnamon in others (Pl IV F and fig 51 A) submerged mycelium persistently colorless in some strains developing many shades of yellow orange orange brown to red (Madder brown of Ridgway Pl XIII) or almost black in reverse of colonies in others in some strains producing at the surface of the agar closely woven yellow to orange masses of hyphae enmeshing numerous helicoid or variously twisted thick walled hulle cells occasional strains showing dark masses

suggestive of sclerotia aerial mycelium more or less abundant colorless or yellow to orange, producing in many strains numerous large drops of transpired fluid, pale to yellow or orange red (acting as an indicator, changing from yellow with acid to orange red with alkali) commonly

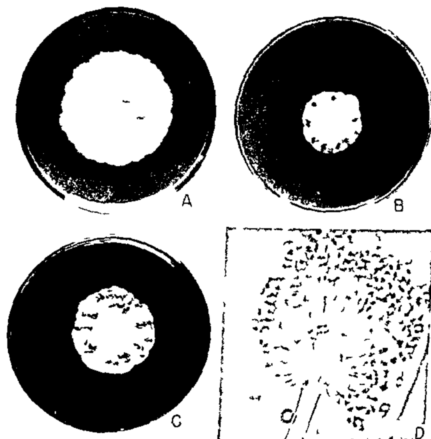


FIG 51 *Aspergillus flavipes* group *A A flavipes* NRRL No 295 growing on Czapek s solution agar 2 weeks room temperature *B* Strain NRRL No 287 of the same species found by White (1943) to produce a penicillin like substance *C* Strain NRRL No 1959 characterized by the production of an excessive amount of exudate *D* Photomicrograph of a typical head showing the elongate vesicle and crowded sterigmata in two series × 750

characterized by a disagreeable odor approaching putridity Heads typically becoming columnar masses shading from persistently white, through shades of pale to deep avellaneous as in *A terreus* but usually in rather sharp contrast to the color of the mycelium Conidiophores from 300 to 500μ by 4 to 5μ in crowded areas up to 2 to 3 mm in length and

8 to 10μ in diameter in some strains and under some conditions of culture walls more or less yellow under the microscope with color mostly localized in the outer layers of the cell wall and occasionally as disk like concretions on the surface of walls otherwise smooth Vesicles subglobose to elliptical (fig 51 D) up to 30 by 40μ in the largest forms usually with diameter twice that of the conidiophore in smaller forms Sterigmata in two series colorless or nearly so closely packed over the apex of the vesicle in small heads and covering the vesicle in large heads primary sterigmata about 6 or 8μ by 2 to 3μ secondary sterigmata 5 to 8μ by 1 5 to 2μ Conidia 2 to 3μ smooth subglobose colorless or nearly so under high magnification with chains aggregated to form columns as seen with the handlens in old cultures

Cosmopolitan in distribution and particularly common in soil and upon decomposing organic materials

Historically the name applied to the series is taken from strains 4640 474 and 4640 402 (Thom Collection) obtained through daFonseca from the Bainier collection in Paris in 1922 These strains showed smooth yellow conidiophores 300 to 400μ by 3 to 4μ contrasting with heads that were rather persistently white and possessed the general morphology of the series as described above Hülle cells were not found Nevertheless the close relationship of these organisms to a great series of cultures obtained from many sources in which these structures are regularly found justifies us in broadening the use of the name

Culture No 4640 486 (Thom) received from the Bainier collection as *S rubescens* (NRRL No 291) shows deep floccose colonies with few heads and scattered dark hyphal masses in age Hülle cells have not been seen in this strain

Among forms commonly obtained from soils collected from widely scattered areas in this country and abroad a series of isolates seems to comply with the description given by Blochwitz for *Aspergillus archi flavipes* (Ann Mycol 32(1/2) 84 1934) This species as described represents an extreme development toward radiate heads abundant conidia conidiophores 2 to 3 mm in length and the development of deep brown or actual red shades of color in the mycelium Several strains observed in culture approach this description (fig 51 C) Heads are at first globose then become slowly barrel form i e short stocky columns Large brown drops of transpired fluid are commonly seen which become yellow when acidified and return to reddish shades when alkali is added Recognition of the species does not appear warranted since no clearly definable character exists which distinguishes these isolates from the less colored forms generally considered as representing *Aspergillus flavipes* in a more restricted sense

Antibiosis

Working with a culture of *Aspergillus flavipes* from Thom (No 4303 46), NRRL No 287, White (1943) has recently demonstrated the production of an antibacterial substance which in its action against *Staphylococcus aureus* strongly resembles penicillin The substance was produced in greatest amount in a medium containing 5 to 10 percent corn steeping liquor as the sole nutrient The addition of sugar is reported as definitely deleterious

Occurrence and Economic Importance

Members of the *Aspergillus flavipes* group like those of the *A ustus*, *versicolor*, and *terreus* groups are cosmopolitan in distribution and are especially common in fertile soil and upon decaying vegetation They are not known to be active agents of decomposition but are capable of growing in the presence of a limited amount of water, hence are probably significant in initiating or continuing processes of decay where most micro-organisms are incapable of growing Little is known of the biochemical activities or products of these forms

Chapter XIV

THE ASPERGILLUS VERSICOLOR GROUP

Outstanding Characters

Conidial heads hemispherical to almost globose in many different shades but usually showing green or blue green
Conidiophores smooth, colorless, more or less sinuous
Vesicles globose to ovate or elliptical with radiate sterigmata borne over the upper half to three fourths of the surface
Sterigmata in two series
Spores globose or subglobose echinulate
Hulle cells found in occasional strains globose

Members of the *Aspergillus versicolor* group are cosmopolitan in distribution They occur regularly in soil, upon decaying vegetation upon stored grains upon cured meats and upon a multitude of other products exposed to occasional moist air or undergoing slow decomposition The morphology of all strains (with the exception of *Aspergillus janus*) is basically alike but different strains vary tremendously in their cultural appearance This is especially true of *Aspergillus versicolor* where conidial heads in different strains vary in color from dark blue-green through green to yellow-green to yellow and orange and finally in some strains to yellowish-cream buff or flesh color

Group Key

I Heads typically globose less commonly hemispherical blue green in color with the blue element dominant colony reverse and substratum usually in red or maroon shades *A sydowi* series
  A Conidial heads always blue green globose to radiate *A sydowi* (Bain and Sart) Thom and Church
  B Conidial heads of two types (a) blue green in color vesicles subglobose to elongate borne on short conidiophores and (b) white clavate borne upon long conidiophores *A janus* Raper and Thom

II Heads hemispherical or nearly globose at times in green shades without blue admixture buff to orange yellow or occasionally flesh colored colony reverse usually in pink yellow red or purple red shades rarely almost colorless *A versicolor* series
  A Conidia echinulate *A versicolor* (Vuill) Tiraboschi
  B Conidia smooth *A humicola* Chad and Sach

Aspergillus sydowi (Bain and Sart) Thom and Church The Aspergilli, p 147 1926

Synonym *S sydowi* Bainier and Sartory, in Ann Mycol 11 25–29, Pl III 1913 Compare Pl IV cultures No 4235 17 and K22, Thom and Church The Aspergilli 1926

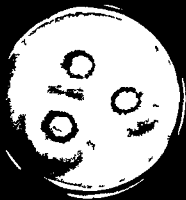
Colonies upon Czapek's solution agar growing well at room temperature, in most strains close textured and velvety from crowded conidiophores and heads arising from the substratum (fig 53 A), in other strains more or le s



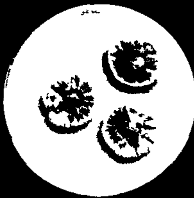
FIG 52 Conidial structures in *Aspergillus sydowi* NRRL No 250, × 800 A Typical large conidial head borne upon an erect conidiophore arising from the substratum B Small head borne as a lateral branch on an aerial hypha consisting of a small cluster of double sterigmata C Minute heads in which sterigmata appear in a single series only

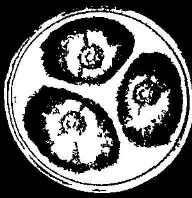
floccose (fig 53 B) from interlacing and trailing aerial hyphae bearing conidial heads ranging from large, well formed structures to minute fruits consisting of clusters of simple sterigmata bearing few conidial chains blue green in color approximately Delft blue or deep Delft blue (Ridgway Pl XLII) with the blue effect especially marked in young fruiting areas (Pl V A) reverse usually in shades of red from coral red to maroon (Ridgway













P V

4 (ppe l ft) *4 pe gill sydow* (B 1 9 t) Th 1 Ch h NRRL N 250 *B* (ppe rigl t *4 pe g ll* us I pe d Th NRRL N 1 8 *C* (ce te left), *A pergill* *m ol* (V ll) T rabos- } NRRL N 239 *D* (ce te n l t) *A pe g ll* *n ol* (V ll) T rabos) NRRL N 233 *E* (l l ft *A perg ll t cr* Th m NRRL N 2 5 (Th colo y colors l l1 be nam lades Th l) t co ld t b ttained k g th col reprod t ns) *F* (l w n l t) *A pe g ll ca* (T gh) Bl t NRRL N 19 8 (Col y col rs h ll b l f eo f w D ffi lt co te l rep ro l g th colors f this fig re) l gure *B* lt re grow g m lt tract g ll tl l res grow g po C pek sol t ga (Col ph tographs by H nes N rth R gional Research Laboratory Reprod ced thro gh co-operatio f Chas Pfize & Co I)

Pls XIII and I) to almost black in some strains in age Conidial heads typically radiate to nearly globose (fig 52 A) ranging from 100 to 150μ but often reduced to small penicillate clusters of sterigmata especially in marginal colony areas and upon aerial hyphae (fig 52 B and C) Conidio-

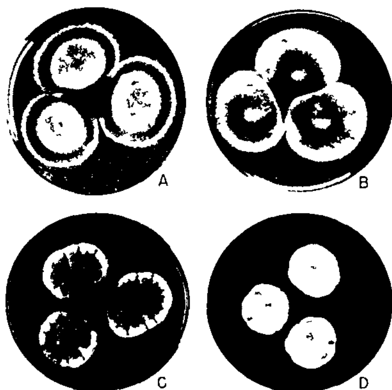


FIG 53 Colony types in the *Aspergillus versicolor* group on Czapek s solution agar, 10 days room temperature *A* Typical heavy sporing strain of *A sydowi* *B* Floccose lighter sporing strain of the same species *C A versicolor* NRRL No 239 characterized by abundant green conidial heads and the production of deep red exudate *D A versicolor* NRRL No 2?7 characterized by flesh-colored conidial heads

phores mostly arising from submerged hyphae up to 500μ in length by 5 to 8μ in diameter colorless smooth comparatively thick walled Vesicles nearly globose fertile over almost the entire surface up to 20μ in diameter Sterigmata in two series primary 6 to 7μ by 2 to 3μ secondary 7 to 10μ by 2 to 2 5μ Conidia globose 2 5 to 3 0μ (Bainier) in our culture up to 3 5μ in diameter conspicuously spinulose green en masse Globose hulle cells

closely resembling those of the A nidulans group have been seen in occasional strains No sclerotia or perithecia are found, but clamydospores in solid substrata are reported

A typical member of our series of isolations was sent to Bainier who concurred in our interpretation of his species

Reduced conidial apparatus appears in varying degree in all strains of *A sydowi* examined In typical strains primary sterigmata with their *clusters of secondaries each bearing* a chain of conidia are found singly or variously grouped along trailing aerial hyphae In other strains there is a progressive development of aerial mycelium in the form of trailing hyphae either single or in ropes, coupled with a reduction in the number of typical *A sydowi* heads Strains are even occasionally seen in which only a few *A sydowi* conidiophores and heads are found in what is otherwise a penicillium like colony Thus a series of strains exists which shows a fairly complete *gradation from Bainier and Sartory's Sterigmatocystis sydowi* to *Penicillium restrictum* of Gilman and Abbott We are led to believe that the latter species should probably be assigned to this section of the genus *Aspergillus*

While morphologically very close to *A versicolor, A sydowi* is easily presumptively recognized by the characteristic blue green color of its conidial heads and the red colors in the substratum A partial list of the sources of *the isolations studied includes soil in Washington* D C, *Illinois* Manitoba, Florida and Ceylon, moldy silk from a stocking factory, concentrated sugar products from Louisiana, dried fish in Japan and bee hives in Michigan It is world wide in distribution and very adaptable to substrata of widely different nature

Probable Synonyms

Aspergillus tiraboschii Carbone (Atti d Inst Bol Univ Pavia Ser II Vol XIV p 320 1914) is described in the colors of *A versicolor* but with the head of *A sydowi* It would appear to be more or less intermediate but unless reisolated must be dropped because of incomplete data

Sterigmatocystis tunetana Langeron (Bull Soc Path Exot 17 345–347 text fig 1924) This mold was recorded as isolated *from an ulcer of the hand but failed* to produce lesions in animal tests as described the colonies were blue green as in *A sydowi*

A sydowi var *achlamydosporus* Nakazawa Simo and Watanabe (Jour Agr Chem Soc Japan 10(2) 178–179 1934) The absence of chlamydospores (hülle cells ?) in a strain of *A sydowi* is hardly a sound basis for separation

Sterigmatocystis cyaneus Mattlet (Ann Soc Belg Med Trop 6 32 1926) was described without data to separate it from *A sydowi*

S cameleo Sartory Sartory and Meyer (Ann Mycol 29 360–361 Pl III figs 7 8 1930) by description must have been some strain of *A sydowi although the very small* smooth conidia do not agree It may possibly represent a form near *A humicola* as described by Chaudhuri and Sachar (see p 193)

Aspergillus janus Raper and Thom in Mycologia 36 556–561, fig 1 1944

Species characterized by conidial heads of two distinct types (1) large white heads borne upon long conidiophores terminating in strongly clavate vesicles and (2) smaller dark green heads borne upon short conidiophores with typically ovate vesicles (Pl I E and F, and V B)

Colonies varying greatly in color and in texture depending upon the substratum and the temperature of incubation Upon Czapek s solution agar at 24 C (fig 54 A) colonies spreading irregularly usually consisting of a central floccose mass 1 to 2 mm deep pale yellow buff in color bearing few and scattered fruiting structures surrounded by an irregular zone of crowded fructifications with dark green heads occurring in a dense stand adjacent to the substratum (fig 54 D) and with numerous long stalked white heads projecting above this layer (fig 54 C) reverse in dull yellow to light brown shades When incubated at 20 C colonies more restricted, less floccose and consisting almost exclusively of a dense stand of long stalked white heads with small green heads absent or developing only in age and arising from trailing aerial hyphae entwined among the white fruiting structures When incubated at 30 to 32 C colonies close textured predominantly green but with central area commonly showing irregular patches of massed hülle cells buff to dull yellow in color Conidial heads abundant and consistently dark green in color Reverse in dull brown shades

White conidial heads loose in texture (fig 54 C) consisting of radiating and divergent chains of conidia commonly 150 to 200µ in diameter occasionally larger Conidiophores long thin mostly 2 to 2 5mm in length by 8 to 10 5µ in diameter occasionally larger, erect essentially uniform in diameter throughout but often marked by numerous and irregularly spaced constrictions walls smooth colorless approximately 1 to 1 4µ in thickness Vesicles thin walled clavate (fig 54 G) mostly 45 to 60µ by 15 to 18µ with individual structures larger or smaller entire surface loosely covered by sterigmata as a rule but often showing barren areas which may occupy any part of the sterigmatic surface Sterigmata in two series primaries 7 to 10µ by 3 5 to 4 5µ secondaries 6 to 8µ by 2 5 to 3µ Conidia smooth, colorless globose to subglobose mostly 2 to 2 5µ with maximum about 2 8µ

Green conidial heads compact radiate when young becoming columnar in age and often spreading into two divergent columns Heads at first in blue to blue green shades near dark gobelin blue (Ridgway Pl XXIV) becoming dark olive gray in age (Ridgway Pl LI) in size commonly ranging from 60 to 75µ in diameter to 200 to 300µ in length Conidiophores erect commonly 300 to 400µ in length by 6 5 to 8µ in diameter of uniform thickness throughout walls smooth, colorless or very faintly green approximately 1 to 2µ thick enlarging rather abruptly into an ovate vesicle Vesicle thin walled variable in form and dimensions but commonly ovoid (fig

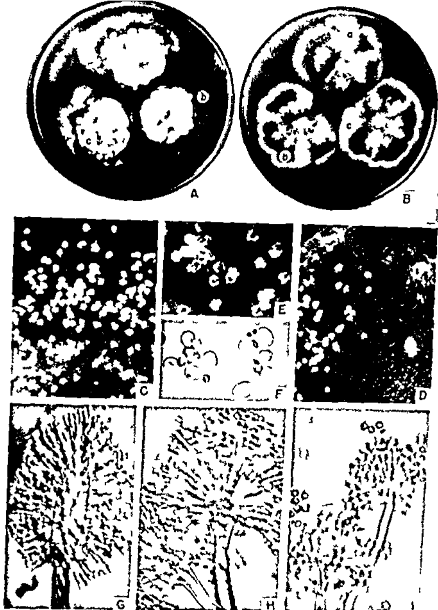


Fig 54 *Aspergillus janus* NRRL No 1787 *A* and *B* Colonies on Czapek's solution agar and malt agar respectively showing *a* scattered white conidial heads, *b*, crowded green heads and c massed hülle cells two weeks old incubation at 24° C × ½ *C* Characteristic white heads borne on long thin conidiophores × 7.5 *D* Massed green heads in dense stand adjacent to the substratum with white heads projecting from the left and with localized development of hülle cells in two limited areas at right × 7 5 *E* Heads of mixed character with older (outer) conidia white and younger (inner) conidia green, × 24 *F* Hülle cells × 350 *G* Typical white head showing clavate vesicle double sterigmata and small smooth conidia × 450 *H* Head of mixed character at this stage (early) producing small white smooth conidia × 450 *I* Typical green head showing small nearly globose vesicle double sterigmata and larger echinulate green conidia × 450 (Reprinted from Raper and Thom New Species of Aspergilli from Soil Mycologia 36 Nov-Dec 1944)

54 I) and occasionally conspicuously elongate typically fertile over the entire area ranging in size from 20 to 30µ by 12 to 18µ Sterigmata in two series rather loosely arranged primaries 7 to 10µ by 4 to 4 5µ secondaries 6 to 8 5µ by 2 to 2 8µ Conidia dark green in mass conspicuously spinulose (fig 54 I) globose mostly 2 5 to 3 5µ occasionally larger or smaller

Conidial heads of mixed character containing both white and green spores commonly encountered (fig 54 E) usually borne upon long conidiophores approaching and often equalling in length those of white heads vesicles clavate (fig 54 H) sterigmata at first bearing colorless smooth walled conidia but subsequently bearing dark green spinulose conidia At temperatures of 24° C and above thick walled hülle cells abundant irregular in form (fig 54 F) commonly globose to subglobose not infrequently elongate commonly more or less curved and often lobed

Colonies upon malt extract agar growing luxuriantly (fig 54 B) generally loose in texture with aerial mycelium prominent, conidial heads normally more abundant than upon Czapek agar the proportion of white to green heads varying with the temperature of incubation

Colonies upon hay infusion agar spreading broadly consisting of a thin submerged mycelium from which develop erect white and green conidial structures the relative proportion of these types being dependent upon the temperature of incubation since comparatively meager growth occurs upon this medium and since there is a minimum of aerial vegetative hyphae it constitutes a very favorable substratum upon which to observe the formation of the contrasting fruiting structures characteristic of the species

The binomial *Aspergillus janus* was selected for this species because of the contrasting types of conidial heads produced—it is literally a ' two faced' mold

Type culture NRRL No 1787 was isolated in February 1942 from Panama soil collected during the summer of 1941 by John T Bonner of Harvard University Three additional isolations by members of the Northern Regional Research Laboratory staff have since been made from Panama soils subsequently collected by Mr Benjamin T Coghill

It is believed that this species represents a normal component of the microflora of Panama Additional evidence in support of this view is furnished by the fact that in 1925 Professor Roland Thaxter sent to Thom under the label white Panama Aspergillus a representative of this species The form was never described by Thaxter and viable cultures of it were lost from our collection some time prior to 1930 As the correspondence of the time is remembered Thaxter was plagued by the presence of a small green mold which repeatedly appeared in his cultures as a contaminant ' Fortunately the original tube received from Thaxter has been preserved

and re-examination of this culture leaves no question but that he was dealing with a strain of the species here described and that the green form which troubled him so much was not in fact, a contaminant but a different phase of the same fungus

Aspergillus janus var *brevis* Raper and Thom, in Mycologia 36 561-563, fig 2 1944

The variety differs from the species in a number of particulars foremost among which are (1) the reduced length of the conidiophores bearing both white and green heads and (2) a consistent tendency for white and green conidial structures to develop in approximately pure stands and to appear as contrasting radial sectors

White conidial heads are of the same general pattern and form as in the species but are of somewhat smaller dimensions are borne upon conidiophores generally less than 2 mm in length by 6 to 8µ in diameter and are characterized by elongate but not strongly clavate vesicles measuring 20 to 25µ by 14 to 18µ conidia are smooth walled colorless, globose to subglobose, 2 2 to 2 8µ in diameter Green conidial heads are compact globose to somewhat columnar borne upon conidiophores 75 to 125µ by 4 to 6µ with globose to subglobose vesicles measuring 8 to 15µ by 10 to 18µ, conidia are dark blue green, strongly echinulate and 3 5 to 4 5µ in diameter

The vesicles of white heads in the variety *brevis* are of approximately the same size and form as the vesicles of green heads in the species itself, whereas the conidiophores bearing each type of head are approximately one half the length of those bearing the same type of head in the species The most striking character distinguishing the variety, however, is the manner in which areas of white and green heads are sharply separated along radial lines Conidial heads of mixed character are produced and usually can be found along the frontier between white and green sectors

Type culture NRRL No 1935 was isolated in July 1942 from a sample of soil collected in Alameda in southern Mexico and forwarded to us in June by Mr William B Roos

Aspergillus versicolor (Vuill) Tiraboschi in Ann Botan (Rome) 7 9 1908

Synonym *S versicolor* Vuillemin Mirsky B in Thèse de Méd Nancy no 27 p 15 et seq 1903 See Thom and Church The Aspergilli p 142 1926

Colonies upon Czapek's solution agar rather slow growing, compact in some strains velvety and consisting almost entirely of closely crowded conidiophores arising from the substratum in other strains showing a marked development of floccose hyphae bearing more or less abundant

conidiophores as short aerial branches in still others a combination of both growth types with colony centers initially floccose and outer areas almost velvety at first white passing through shades of yellow orange yellow, tan

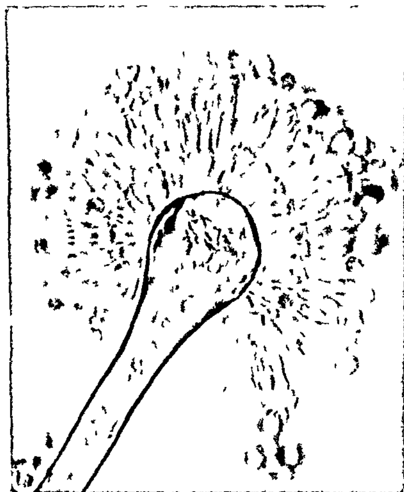


FIG 55 *Aspergillus versicolor* strain NRRL No 2 Photomicrograph showing details of bead structure Note particularly the double series of sterigmata × 2100

to yellowish green shades such as pea green or sage green (Ridgway Pl XLVII) depending upon the strain (Pl V C and D and fig 53 C and D) and conditions of culture occasionally with the green colors almost or com

pletely lacking, reverse and substratum occasionally colorless or nearly so, mostly passing through shades of yellow to orange then rose purple red or red, the particular shade and intensity of color normally persisting as a strain or varietal character *Heads roughly hemispherical radiate* up to 100 to 125μ in diameter, rarely approaching columnar Conidiophores colorless, smooth up to 500 or even 700μ by 5μ or approaching 10μ near the vesicles Vesicles 12 to 20μ in diameter, fertile area hemispherical or semi elliptical (fig 55) passing almost imperceptibly into the funnel like enlarged apex of the conidiophore Sterigmata in two series primary commonly 8 to 10μ by 3μ, occasionally less, secondary 5 to 10μ by 2 to 2 5μ Conidia *globose, usually delicately echinulate, mostly 2 5 to 3μ, occasionally 3 5μ or 4μ,* usually borne in loosely radiating chains

In occasional highly colored strains vesicles and sterigmata may be colored

Hülle cells of the *Aspergillus nidulans* type are occasionally seen

Neither perithecia nor sclerotia have been found

The diagnosis is drawn broadly enough to cover a common and very abundant *series of organisms which vary greatly in colony appearance* In some strains aerial growth is composed of conidiophores and heads only, in others it is made up of floccose felts or ropes of hyphae bearing short conidiophores and small heads which retain the characteristic arrangement of vesicles, sterigmata, and conidial chains

The wide range of colony structure and coloration of strains,[1] and the varied conditions under which these have been collected probably account for the appearance of many names in the literature which refer to cultures of the group but which cannot be safely separated and identified by the de scriptions given

The following list of species are believed to represent synonyms

S ambari Beauregard (Ann de Micrographie 10 255–278 pl 1 1898) Probably one of the series but never identified again

S bicolor J Ray (Rev Gen Bot 9 193–212 245–259 282–304 Pl 12–17 1897) is probably one of this series Primary sterigmata were reported filled with red coloring matter and conidia globose spinulose up to 2.5μ in diameter

Sterigmatocystis brodens Mattlet (Name only in Ann Soc Belge Med Trop 6 31 1926 discussion ibid 4 167–171 figs 1 2 1924) Apparently a member of this series

A flavo viridescens Hanzawa (Jour Coll Agr Tohoku Imp Univ Sapporo 4 232–3 pl 21 figs 1–4 1911) Culture No 4291 10 (Thom) received from Hanzawa under this name belongs to the *A versicolor* group

A versicolor var *glauca* Blochwitz (Ann Mycol 32(1/2) 86 1934) This variety has the color of the *A glaucus* group which is more deeply blue than *A versicolor* Isolated in the skin clinic at Kiel upon human skin showing ringworm

[1] For a more complete discussion of the range of cultural types found in this series the reader is referred to Thom and Church The Aspergilli pp 142–145 1926

S glauca Bainier (Bul Soc Bot France 27 29–30 pl 1 fig 3 1880 ibid 28 77 1881) From extract of henbane dregs of wine casks and corks

A globosus Jensen (N Y Cornell Agr Exp Sta Bul 315 p 482 1912) The type culture received from Whetzel (Thom No 2705) is certainly a member of this series and is characterised by yellowish green to olive green conidial areas with colony reverse in yellowish-orange to wine red

S polychroma Ferraris (Fl Ital Crypt Hyph p 640) Syn *A versicolor* fide Tiraboschi in Ann de Botanica (Rome) 7 9 1908

S spuria Schroeter (Cohn Krypto Fl von Schlesien 3 2 Hälfte Lief 1 p 218 1893) Position in doubt May represent a form of *A versicolor* similar to the flesh colored forms discussed by Thom and Church in The Aspergilli p 145 1926 or may belong with *A carneus* (See p 201)

A tabacinus Nakazawa Simo and Watanabe (Jour Agr Chem Soc Japan 10(2) 177 178 1934) The detailed figures given in contrast to their own strain of *A versicolor* showed differences which disappear when large numbers of isolations are studied

Aspergillus humicola Chaudhuri and Sachar in Ann Mycol 32 97 1934

Characterization after Chaudhuri and Sachar

Colonies on Czapek s solution agar, at first white passing through shades of olive-gray (Ridgway, Pl XLVI 20‴‴) velvety at margin floccose toward the center reverse and substratum in shades of yellow, heads radiate Conidiophores arising directly from the substratum up to 300μ in length by 4 to 5 4μ in diameter or as short branches, about 70μ long from aerial hyphae walls smooth and almost colorless Vesicles 9 to 15μ in diameter, colorless flask-shaped with sterigmata radiating from the whole surface of the larger heads or only borne in the upper third in small heads primary sterigmata 3 6 to 5 4μ by 1 8 to 2μ secondary sterigmata 3 6 by 1 8μ Conidia globose smooth 2 to 3μ in diameter, in radiating chains

Neill (1939) is believed to have correctly placed this organism with *A versicolor* and its allies despite the smoothness of its conidia The almost colorless conidiophore suggests relationship to *Aspergillus ustus* in this group certain forms (e g Blochwitz s *A ustus* var *laevis*) apparently have spores smooth or nearly so (see p 175)

Pathogenesis

Aspergillus sydowi is not reported by name as a parasite but strains described in terms which must be interpreted as placing them with *A sydowi* includes *A tunetanus* (Langeron) Dodge from fleshy lesions on a hand in Tunis *A Vancampenhouti* (Mattlet) Dodge also from tropical Africa *A cyaneus* (Mattlet) Dodge from the same region The evidence at hand links *A sydowi* more closely with *A nidulans* than was formerly supposed although such relationship is strongly indicated by the identical character of their hülle cells The fragmentary descriptions commonly given for

individual pathogenic molds isolated by persons unfamiliar with the literature are rarely definite enough to separate nearly related forms

An occasional culture of *A versicolor* is obtained from apparently pathogenic sources Thus far experimental work has not shown evidence of actual lesions in human flesh Little colonies bearing green heads and conidia were drawn with a breast pump from an inflamed mammary gland and kept in culture for many years but failed to grow in laboratory media at blood heat *Aspergillus versicolor* var *glauca* Blochwitz was isolated from human skin showing ringworm' at the skin clinic in Kiel but apparently pathogenicity was not proved experimentally Strains of *A versicolor* are frequently observed upon dried salted lean beef thus showing its capacity to grow in and upon meat products, but not giving direct evidence of participation in any pathological process

Occurrence and Economic Importance

Members of the *Aspergillus versicolor* group appear widely distributed in soil, on spoiling and drying food stuffs breads, cereals old cheese, dried meats, cured India rubber, musty vegetable products, and other substrata characterized by a moderately low water content or containing factors toxic to most organisms They are reported as capable of decomposing certain paraffins The production of proteolytic enzymes by most strains is shown by the digestion of milk and the liquefaction of gelatin *Aspergillus sydowi*, in particular is a characteristic component of all soil examined

Fat production by *A sydowi* has been studied quite extensively by Professor Peterson and associates at the University of Wisconsin For reference to this work see the various papers listed in the Topical Bibliography under the heading Chemistry of Mold Tissues '

Chapter XI

THE ASPERGILLUS TERREUS GROUP

Outstanding Characters

Heads columnar in cinnamon pale buff or light flesh colors
Conidiophores smooth colorless rarely exceeding 250μ in length
Vesicles hemispherical with upper half to two thirds covered by sterigmata
Sterigmata in two series generally crowded
Conidia smooth globose to slightly elliptical small

Included here are members of a variable and cosmopolitan group of Aspergilli especially common in soil They differ markedly in color and in colony appearance and to a lesser degree in the texture of their conidial heads The group may be separated as follows

Group Key

I Conidial heads in cinnamon or orange brown shades compact uniform in diameter throughout — *A terreus* series
- A Colonies velvety conidiophores mostly in a dense stand arising from the substratum
  - 1 Conidial heads in dull cinnamon shades — *A terreus* Thom
  - 2 Conidial heads orange brown near xanthine orange (Ridgway) — *A terreus* var *boedijni* (Bloch) n var
- B Colonies floccose conidial heads arising from aerial hyphae
  - 1 Mycelium colorless heads light pinkish-cinnamon in color — *A terreus* var *floccosus* Shih
  - 2 Mycelium yellow heads developing late in cream or light tan shades — *A terreus* var *aureus* n var

II Conidial heads white or flesh colored loose textured not strictly uniform in diameter — *A carneus* series
- A Colonies in light flesh colors ranging from near white to vinaceous fawn (Ridgway) Thick walled hyphae suggestive of hülle cells are generally present — *A carneus* (van Tieghem) Blochwitz
- B Colonies persistently white or becoming dull ivory in age Thick walled cells often present — *A niveus* Bloch

Aspergillus terreus Thom in Turesson Gote Svensk Botanisk Tidskrift 10 5 1916 without description diagnosis Thom and Church in Amer Jour Bot 5 85–6 1918

Synonym *A galeritus* Blochwitz in Ann Mycol 27(3/4) 205 Taf III 1929

Colonies upon Czapek s solution agar growing well at room temperature and up to 37 C spreading plane or marked by shallow radial furrows

Fig 56 *Aspergillus terreus* *A* Colony margin of a typical strain NRRL No 265 showing crowded long columnar heads × 14 *B* Scattered but typical conidial heads of an ultra violet light produced mutation unable to utilize NO_3 nitrogen × 50 Both cultures on Czapek s solution agar 2 weeks

velvety (fig 58 A) or in some strains showing tendency toward floccosity in central colony areas heavy sporing throughout with massed columnar heads giving to colonies their characteristic color and texture, in color ranging through various cinnamon shades (Ridgway, Pl XXIX) depending upon the abundance and maturity of the heads (Pl V E) amber exudate produced in some strains odor transient to none reverse in dull yellow to brown shades Conidial heads long columnar (fig 56 A) with conidial chains compacted together of uniform diameter throughout entire length commonly ranging from 150 to 500μ or more in length by 30 to 50μ at maturity, ranging from cinnamon buff through cinnamon to Sayal brown (Ridgway Pl XXIX) Conidiophores more or less flexuous smooth, colorless commonly ranging from 100 to 250μ by 4 5 to 6 0μ approximately uniform in width throughout (fig 57 A and 19 A) Vesicles hemispherical dome like commonly 10 to 16μ in diameter merging almost imperceptibly into the supporting conidiophore Sterigmata in two series primaries crowded (fig 57 A) parallel 5 0 to 7 0μ by 2 0 to 2 5μ secondaries closely packed 5 5 to 7 5μ by 1 5 to 2 0μ Conidia globose to slightly elliptical commonly 1 8 to 2 4μ in diameter

Species description based upon type strain NRRL No 255 (Thom No 144) and innumerable additional isolations from soils and other sources in this country and abroad The species is especially abundant in warm and comparatively dry arable soils

The great majority of isolates belonging to this series fall within *A terreus* in its strictest sense and duplicate in all essential particulars the description given above for this species Nevertheless wide natural variation among strains is encountered when great numbers are isolated from widely separated sources Some of these are quite striking in appearance and have apparently furnished the bases for species and varietal description by other workers

Aspergillus terreus var *boedijni* (Bloch) n var

Blochwitz in Ann Mycol 32(1/2) 83 1934 described *Aspergillus boedijni* as a new species differing from *Aspergillus terreus* primarily in the color of its conidia These were reported as pure yellow at first becoming pure brown or ochraceous brown in age and brighter than *A galeritus* Blochwitz[1] In our experience strains are occasionally encountered which are characterized by a bright orange brown color instead of the dull cinna

[1] *A galeritus* Blochwitz (Ann Mycol 27(3/4) 205 Taf III 1929) is a redescription of *A terreus* Thom Blochwitz acknowledged having Thom s type at hand when he renamed this species No reason was given beyond the claim that he had had the organism in culture for some years before Thom s description of *A terreus* was published

mon shades typical of the species Among cultures currently under examination, this character is noted in an isolate from Argentine soil is somewhat more marked in NRRL No 680 from Dr G A Ledingham Ottawa, Canada and is particularly striking in NRRL No 1913 isolated by Dr C W Emmons from Arizona soil In all of these strains the basic morphology is that of a typical *A terreus* hence Blochwitz's separation is reduced to varietal rank

Aspergillus terreus var *floccosus* Shih, in Lingnan Sci Jour *15* 372, Pl 16 fig 3 1936

Strains characterized by deep floccose colonies in which conidial heads are less abundant develop late and are borne almost entirely upon aerial hyphae are frequently encountered (fig 58 B) While the conidial structures of certain of these strains appear entirely normal in the majority of isolates the heads are somewhat less compact and generally lighter in color This color difference is particularly marked among isolates from soils collected in Texas Central America and Cuba with color commonly light pinkish-cinnamon (Ridgway Pl XXIX) to vinaceous buff (Pl XL) in age No sharp line of separation can be drawn between typical strains of *A terreus* and the floccose forms under consideration since isolates of intermediate character are encountered, nevertheless these strongly floccose cultures occur with sufficient frequency to warrant recognition of Shih's varietal designation if his interpretation is somewhat broadened The variety is considered by the writers as a strongly floccose *Aspergillus terreus* in which the head is commonly less compact and lighter in color, but with the basic morphology of the conidial apparatus remaining that of the species proper This variety is represented by such strains as NRRL Nos 1920 and 1921 isolated from Cuban soil contributed by Professor J M Osorio University of Havana, No 1922, isolated from Texas soil collected and sent to us by Dr F E Clark from Greenville Texas

Other strains examined almost completely bridge the gap between the pale colored strains of *A terreus* var *floccosus* and the light flesh colored forms characteristic of *Aspergillus carneus*

Aspergillus terreus var *aureus* n var

This new and striking variety differs from the species in a number of particulars colonies upon Czapek's solution and malt extract agars are comparatively slow growing, floccose, ranging up to 3 to 4 mm deep, and are bright golden yellow in color Conidial structures are produced tardily and in limited numbers Conidiophores are appreciably longer than those of the species often becoming 500µ or more in length and bear columnar heads generally loose in texture ranging in color from cream or light buff

to light pinkish-cinnamon Microscopically the conidial structures approximate those of the species itself Separation as a new variety is based primarily upon the characteristic coloration of the growing colony

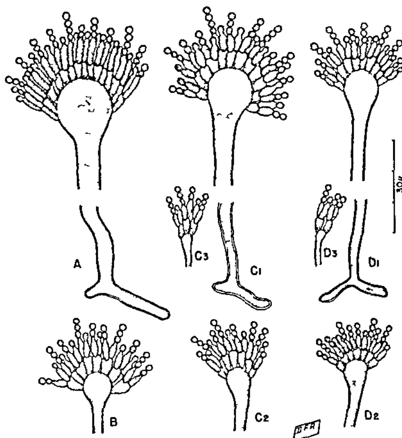


FIG 57 Conidial structures of members of the *Aspergillus terreus* group × 840 *A A terreus* type strain NRRL No 255 (Thom No 164) *B A terreus* var *aureus* NRRL No 1923 C_1 C_2 and C_3 *A carneus* NRRL No 1928, conidial heads vary greatly in size *D D* and *D A niveus* NRRL No 515 conidial heads of varying dimensions

Type strain NRRL No 1923 (fig 16 D) was isolated from Texas soil contributed by Dr F E Clark Additional strains showing approximately the same cultural and morphological characteristics have been isolated from soils collected in Arkansas and Arizona In *A terreus* var *aureus* the yellow coloring matter is lodged in the vegetative mycelium and there are no suggestions of hülle cells in *A carneus* however approximately the same

yellow tints are developed through the massing in localized colony areas of thick walled hyphae suggestive of hulle cells (see p 201)

At least two other species have been described which are believed to represent probably synonyms of *Aspergillus terreus*

Aspergillus fuscus Amons (Archief voor de Suikerindustrie in Nederlandsch Indie Jaarg 29 Deell pp 8–10 1921) by description is obviously a form closely related to if not identical with *A terreus*

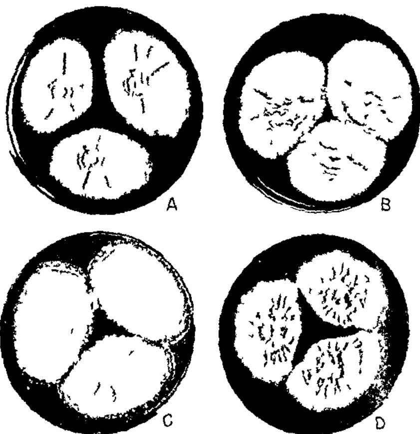


Fig 58 *Aspergillus terreus* group different species growing upon Czapek s solution agar at room temperature *A* Typical, heavy sporing strain of *A terreus* NRRL No 265 *B A terreus* var *floccosus* characterized by loose floccose colonies and limited spore production *C A carneus* NRRL No 1923 heavy sporing and characterized by flesh colored conidia *D A niveus* NRRL No 515 characterized by white conidial heads

Aspergillus cinnamominus (Weiss) Dodge in Med Myc p 677 1935

Synonym *S cinnamominus* Weiss in Ann Parasitol Hum Comp 8 159–193 5 figs 1930

Characterization from Dodge Hyphae septate and branched conidiophores simple 6μ in diameter vesicle 12 by 9μ Primary phialides cylindric 4μ long bearing

2 to 3 sometimes 4 secondary phialides 5 to 6μ long Conidia spherical 1 to 2μ in diameter brown Other spores borne laterally on short branches (phialides ?) 3 to 4μ Chlamydospores occasional Under some conditions only monstrous phialides are formed with a pleomorphic mycelium resulting Described as present in lesions of pityriasis versicolor flava Inoculation into the human skin reproduced the original disease From the description given the organism is some strain of the *A terreus* group

Aspergillus carneus (v Tiegh) Blochwitz

Synonyms *Sterigmatocystis carnea* v Tiegh in Bull Soc Bot France 24 103 1877 Cited also in Saccardo Sylloge 4 74 and in Wehmer's Monograph p 127 (Mem Soc Phys Hist Nat Gen pp 1–157 1899–1901)
Sterigmatocystis spuria Schröter as a bibliographic change in Cohn Krypt Fl Schleisen 3 2 Helfte Lief 1 p 218 1893
Aspergillus carneus Blochwitz in Ann Mycol 31(1/2) 81 1933

Colonies upon Czapek s solution agar growing well at room temperature spreading plane or radially furrowed more or less floccose at first white but becoming pale vinaceous fawn to vinaceous fawn (Ridgway Pl XL) with the development of mature fruiting structures (Pl V F and fig 58 C) ranging from 1 to 2 mm or more deep in central areas to very thin and spreading at colony margin comparatively heavy sporing throughout with fructifications arising from both aerial and submerged mycelium some strains showing limited areas yellow in color from an underlying felt of heavy walled sterile hyphae suggestive of hülle cells (fig 49 F) odor often pronounced somewhat putrid reverse in orange-yellow bright orange, to deep brown shades conidial heads loosely columnar averaging 150 to 200μ by 25 to 35μ but commonly somewhat larger varying in color as the colony Conidiophores variable in length mostly 250 to 400μ but ranging up to 1 mm occasionally bearing secondary fruiting structures as short and irregularly placed branches smooth sinuous uncolored mostly 3 5 to 6 0μ in diameter Vesicles hemispherical, ranging from 5 5 to 9μ rarely as much as 10μ (fig 57 C_1) Sterigmata in two series, primary 5 5 to 6μ by 2 to 2 5μ secondary 5 to 5 5μ by 1 8 to 2μ commonly very few primary sterigmata present Conidia globose to subglobose thin walled averaging 2 4 to 2 8μ with maximum rarely exceeding 3 2μ

Colonies upon malt extract agar growing more restrictedly heavier sporing with pigmentation generally more pronounced and with conidial heads averaging slightly larger than on Czapek s solution agar otherwise duplicating the above description

This species is represented by strains NRRL No 527 isolated as an air contaminant in Washington NRRL No 298 isolated from Kansas soil, and other soil isolations from different parts of the United States Mexico

Cuba, and Central America Strains differing from the above in the absence of any yellow, thick walled hulle like cells are occasionally isolated from soils NRRL No 1928, isolated from Arkansas soil, is representative In the absence of any yellow component, the color of these strains is more accurately described as pale to light grayish vinaceous fawn (Ridgway, Pl XXXIX)

The name *Aspergillus carneus* is revived to cover the forms under consideration since their most obvious identifying characteristic is the pale flesh color of their massed conidial heads It is the belief of the writers that van Tieghem probably had in hand some member of this series when he proposed the name *S carnea*, although due to the inadequacy of his description it is now impossible to establish this point with certainty In any case, the name is excellently descriptive of strains commonly encountered, hence its application in this connection

In describing *Aspergillus carneus* as a new species, Blochwitz (1933) acknowledged the earlier use of this specific name by van Tieghem but disregarded its validity He undoubtedly applied it to a member of the species as it is considered by us since he noted that it differed from *A terreus* (*A galeritus*) principally in the flesh to rose color of its conidia It is believed that Blochwitz's *A niveus* var *nubila* (1934) likewise represented a strain of *A carneus* characterized primarily by conidia of darker rose a condition which in older cultures is frequently suggested by NRRL No 1928 cited above

Gilman and Abbott in their "Summary of Soil Fungi" (1927) called attention to the repeated isolation from Louisiana soils, of forms with the "general morphology of the *Aspergillus candidus* group but producing bright pink conidial heads" While the writers think the affinities of these forms lie more with *Aspergillus terreus* (columnar heads, colorless conidiophores) and *Aspergillus flavipes* (elongate irregular hulle cells) than *A candidus* we have every reason to believe they were dealing with forms similar to those here designated *A carneus*

S albo rosea Sartory Sartory and Meyer (Ann Mycol 28 358–359 Pl III fig 1–6 1930) apparently represents a member of this series This is indicated by the described coloration of colonies and more particularly by the detailed measurements cited for it

Aspergillus niveus Blochwitz, in Ann Mycol 27(3/4) 205–6 fig 2 Taf III 1929

Synonym *A eburneus* Biourge name attached to a culture received by Thom (No 5402 1 NRRL No 515)

Colonies upon Czapek's solution agar white, plane or radially furrowed rather slow growing forming a dense felt of mycelium and conidiophores up

to 600μ to 1 mm deep thinning toward the margin (fig 58 D) and commonly spreading in unevenly radiating lines commonly producing abundant amber to brown exudate reverse in dark yellow shades through greenish to brownish black odor slight Conidiophores smooth with walls colorless sinuate more or less septate slender 4 to 6μ in diameter, enlarging to a hemispherical vesicle 8 to 15μ in diameter or sometimes larger at the apex commonly 300 to 600μ in length occasionally up to 1000μ long and on other substrata sometimes longer Conidial heads showing chains of conidia in comparatively loose columns most frequently 20 to 30μ in diameter but in large heads up to 60μ with the general appearance of a snow white *A terreus* Vesicular area hemispherical (fig 57 D) Sterigmata in two series primary sterigmata 5 to 8μ by 2.5 to 3.0μ secondary sterigmata 5 to 7μ by 2 to 2.5μ Conidia 2.0 to 2.5μ rarely more smooth thin walled colorless

Represented in the NRRL collection by Nos 515 (Thom No 5402.1) 288 and 1955 Repeatedly isolated from soil but less common than *Aspergillus carneus* and the ubiquitous *A terreus*

Typically the conidial apparatus is that of a white loosely columnar form of *A terreus* As described by Blochwitz it does not show yellow in culture This is true of strain NRRL No 515 although in age this culture reaches dull ivory to pale buff on agar slants In other strains such as NRRL No 1955 from Dr Timonin in Ottawa Canada limited areas may become yellow from the development of masked thick walled hyphae as in *A carneus*

Sterigmatocystis pusilla Peyronel (I germi atmospherici dei funghi con micelio Thesis Padova 1931 p 21) probably represents a synonym of *A niveus* Blochwitz It was described as follows Colonies white very thin sterile hyphae creeping sparingly branched falsely septate hyaline 1.5 to 5μ in diameter conidiophores erect unseptate hyaline 60 to 80μ by 2.5 to 3μ with apical vesicles hyaline obovoid or subglobose 7 to 10μ in diameter sterigmata radiate in two series with primaries 5 to 7μ by 2 to 2.5μ and secondaries 3 to 5μ by 2μ in groups of 2 to 3 conidia globose 2 to 2.5μ hyaline smooth Habitat from air in northern Italy at altitude of 1 700 meters From description this would appear to represent a short stalked member of *A niveus*

In their most typical manifestation the conidial heads of *Aspergillus niveus* are loosely columnar vesicles are dome like and fertile over the upper one half to two thirds only They thus stand out in sharp contrast against the typically globose heads and completely fertile vesicles of *A candidus* But all heads of *A candidus* are not globose and all vesicles are not fertile over their entire surface In most strains small columnar heads not particularly different from those of *A niveus* can be found (fig 60 D) Considering this character together with (1) the snow white heads (2) the smooth colorless conidiophores and (3) the small smooth conidia of both species one can readily imagine that we are here dealing with two interlock

ing groups. One basic character separating the two is the production of sclerotia. Characteristic reddish purple to black sclerotia are commonly found in white spored strains producing wholly or in part large globose heads (*A. candidus*), they have never been seen in white spored strains producing only loose columnar heads (*A. niveus*). Until additional intermediate forms are isolated and studied, the relationship between these white spored forms must remain a matter of conjecture, although in this manual they are placed adjacent in what we believe to represent a natural placement of the different groups.

Pathogenesis

Strains of *A. terreus* grow under a wide range of temperature including 37° C. It is not surprising, therefore, that an occasional member of the series is reported as a human parasite. One of these was re-described as *Sterigmatocystis hortai* by Langeron (1922). This culture, NRRL No. 274 (Thom No. 5071.1), received from France, was originally isolated from a human ear in Brazil. It is believed to be type and represents a characteristic strain of *A. terreus*. Another was found in metastatic lesions on a corn husker's hand and forearm in Nebraska. Recently a strain was isolated from an aborted fetus from a cow in Maryland, the culture was entirely typical of *A. terreus*.

Occurrence and Economic Importance

Members of the *Aspergillus terreus* group are typically soil organisms, hence are most abundant in soil and upon decaying vegetation. They frequently occur, however, upon a great variety of materials useful to man, including grains in storage, straw and forage products, cotton and other fibrous materials not adequately protected from excessive moisture, etc. *Aspergillus terreus* and *Aspergillus carneus* are especially widespread in warm arable soils, and have been isolated in great abundance from soils collected in southern and southwestern United States. They are generally less common in forest than in cultivated soils, and are rarely found in acid forest soils from the colder temperate zone. There is little evidence that these forms are especially active agents of decay, but their great abundance in nature indicates that they undoubtedly play a significant role in the slow decomposition of organic materials. *Aspergillus terreus* and *A. carneus* grow well at temperatures of 35 to 37° C, a character which possibly accounts for their great abundance in southern soils and their relative scarcity in soils from northern areas.

Aspergillus terreus has become of special biochemical interest since the discovery in 1939 by Calam, Oxford, and Raistrick that certain strains of this species are capable of producing itaconic acid from sugars. Extensive

investigations on this fermentation have been conducted in the Fermentation Division of the Northern Regional Research Laboratory, and papers reporting these studies are currently in press More than 300 strains have been tested and the most productive cultures selected for intensive study Improved nutrient solutions have been developed and critical environmental factors explored with the result that substantial yields of itaconic acid are now obtained It is believed that this process may become of industrial importance within a reasonable period of time (See Lockwood Raper, Moyer, and Coghill Lockwood and Reeves Lockwood and Ward, Moyer and Coghill, and Raper Coghill and Hollaender, in the Topical Bibliography under Itaconic Acid')

Timonin (1942) reported the production of citrinin by a white-spored Aspergillus identified by him as one of the *A candidus* group Careful examination and comparison of his culture with representative strains of *A candidus A carneus* and *A niveus* show its true relationship to be with *A niveus* in the *terreus* group as it is considered here rather than with *A candidus*

Chapter XVI
THE ASPERGILLUS CANDIDUS GROUP

Outstanding Characters

Conidial heads persistently white or becoming yellowish cream in age, typically globose but approaching columnar in small heads
Conidiophores smooth, colorless or slightly yellowed in terminal areas
Sterigmata in two series with primaries often much enlarged sometimes varying greatly in size within the same head
Conidia globose or subglobose smooth
Sclerotia present in some strains dark, approaching purple to black when mature

Grouping by color lead Thom and Church (1926) to establish their Section IX or the so-called "White spored Aspergilli" Only vaguely did they indicate that they had included a heterogeneous lot rather than described a natural group Further study of the strains included in the 'white' section showed the tardy development of colors approaching avellaneous or even *carneus* *The continued comparison of great numbers of strains* in all groups has revealed such wide variations in color that the authors *have come to regard whiteness or lack of color, as a character of secondary* importance in the allocation of strains to particular groups This view point is supported by the appearance under controlled conditions of white variants or mutants in a number of colored series Yuill (1939) observed and isolated such colorless mutants from *Aspergillus fumigatus* and *A nidulans*, Steinberg and Thom (1940) reported the same type of mutant for the former species, while Raper, Coghill and Hollaender (in press) have succeeded in producing white mutants in *Aspergillus terreus* by irradiating spores with ultra violet Colorless members of the *Aspergillus glaucus* group represented by *A niveo-glaucus* have been isolated by Blochwitz Thom and Raper and other investigators Long before the work of Yuill Schiemann (1916) had developed two color variants of *Aspergillus niger A schiemanni* (Schiemann) Thom (1926 p 172) and *A cinnamomeus* Schiemann (1912) which differed only from the parent strain by a progressive reduction of the amount of coloring substance, presumably the aspergilline of Linossier (1891) Steinberg and Thom (1940) working with a strain of *A niger* again produced variants approximating those of Schiemann and Whelden (1940) secured the same by irradiating spores of *A niger* with cathode rays In cultures

collected from nature from world wide sources strains characterized by heads approaching white are occasionally observed in other groups It is apparent then that the capacity to produce a coloring substance while ordinarily inherited or passed on in successive colonies of an organism is not always uniformly maintained

In the present treatment the writers have sought to include within the *Aspergillus candidus* group only such forms as are clearly and clo ely related to it The group is thus limited essentially to a single series containing only one clearly definable species *A candidus* Different isolations vary materially in their general cultural appearance and in the details of their microscopic structure Nevertheless all possess the typically globose white to dull buff or light gray conidial heads the smooth colorless conidiophores and the small smooth colorless conidia

To facilitate recognition of members of the *Aspergillus candidus* group and to assist in the proper assignment of other white or light-colored species and strains a general key covering all of these forms is presented

GENERAL KEY OF WHITE ASPERGILLI

A Heads (large ones) globose or radiate conidiophores smooth walled colorless or yellowed toward the vesicle only sclerotia occasionally seen *A candidus* group

B Heads white hemispherical to columnar conidiophores smooth walled colorless *A niveus* series see p 202

C Heads initially white tending to be columnar conidiophores smooth walled showing some shade of yellow contorted hülle cells usually found *A flavipes* series see p 179

D Heads white borne upon long smooth walled colorless conidiophores terminating in clavate vesicles sterigmata in two series White-spored phase of *A janus* see p 187

E Strains of white Aspergilli possessing the basic characters of their colored counterparts also occur as mutations in the *A fumigatus* *A nidulans* *A terreus* and *glaucus* groups

Aspergillus candidus Link Obs p 16 1809 Thom and Church The Aspergilli p 157 1926

Colonies upon Czapek s solution agar persistently white or becoming cream or yellowish-cream in age (Pl VI A and fig 59) often thin vegetative mycelium often largely submerged surface growth usually consisting of conidiophores and heads and with scanty sterile mycelium or anastomosing ropes of hyphae bearing short stalked fruiting structures sclerotia produced in occasional strains reverse usually uncolored Heads white globose radiate varying in the same culture from large globose masses 200 to 300μ in diameter to small heads often less than 100μ in diameter commonly more or less elongated in heads with incomplete development of sterigmatic surface Conidiophores varying with the strain

in short or dwarf races less than 500μ long, in other strains ranging up to 500 to 1000μ or longer, varying from 5μ in diameter in dwarf forms to 10 to 20μ in long stalked forms, with walls thick, smooth, colorless or slightly yellowed near the vesicle in certain strains in age Vesicles typically

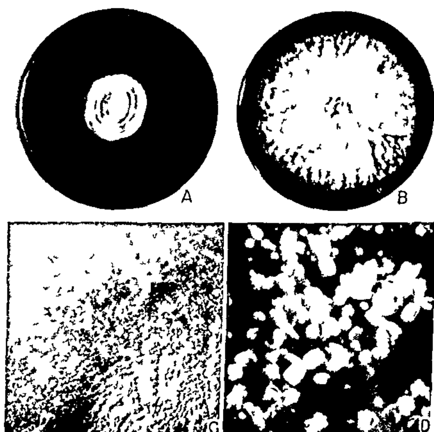
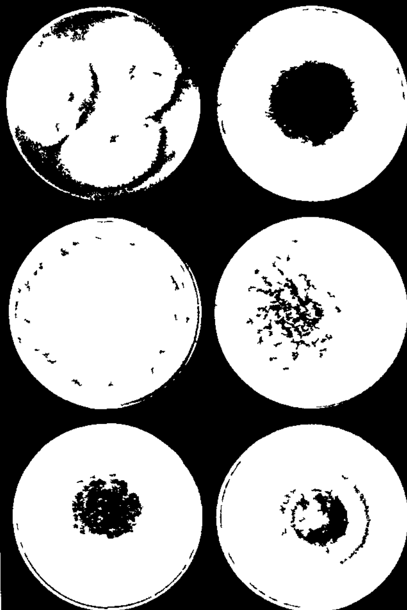


Fig 59 *Aspergillus candidus* A Strain NRRL No 305 on Czapek s solution agar at room temperature 10 days B Strain NRRL No 314 on Czapek s solution agar at room temperature three weeks Note contrast between compact colony of No 305 consisting of crowded conidiophores and loose spreading colony of No 314 in which production of conidial heads is very irregular C Strain NRRL No 312 portion of colony showing scattered black sclerotia × 3 D Strain NRRL No 308 conidial heads × 18

globose, ranging from 40μ in diameter in very large heads (fig 60 A), and typically fertile over the whole surface to small globose heads (fig 60 B) often very much reduced to support simple groups of sterigmata appearing almost penicillate (fig 60 D) Sterigmata typically in two series, usually colorless primary varying greatly in different strains in different heads of



P VI

4 (ppe left) *A pe g ll ca d d* l h NRRI N 308 *B* (ppe n l t) *A pe g ll ger g* p ra NRRL N 6 *C* (ce te l f) T ep red m f tra 6 p od ced by l ra l t rad *D* (ce ter right) *A per ll* th NRRL N 51 *E* l w l ft) *A per ll alliaceus* Tl m d Ch h NRRI N 315 *F* (l n h t *A perg ll* te *l* W h NRRL N 3 5 All lt rea

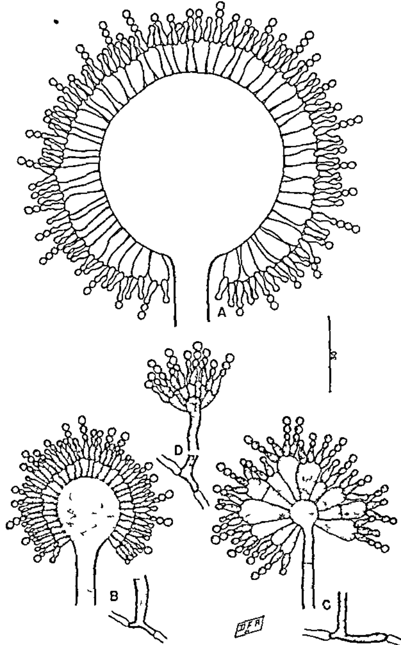


FIG 60 Conidial structure of *Aspergillus candidus* × 900 *A* Large globose head showing large primary sterigmata strain NRRL No 312 *B*, Smaller globose head showing small primary sterigmata strain NRRL No 308 *C* Head showing primary sterigmata of variable size some septate strain NRRL No 312 *D* Diminutive head same strain Great variation in dimension is characteristic of the fruiting apparatus of most *A candidus* strains

the same strain, and occasionally in the same head (fig 60 C) ranging from 5μ in length in some cases to 15 or 20μ and even 30μ under other conditions commonly septate secondary sterigmata usually uniform in all heads from 5 to 8μ by 2 to 2 5 or 3μ Conidia colorless globose or subglobose in most strains to elliptical or barrel form in others, thin walled 2 5 to 3 5μ or occasionally 4μ smooth

Reddish purple to black sclerotia, consisting of thick walled, parenchyma like cells, occur in many strains (fig 59 C)

As there is no possibility of determining which of the white Aspergilli was in Link's possession, the name is here used to cover a whole series of strains which are found everywhere but are most frequent in the later stages of decay in vegetation and are especially characteristic of moldy grain Included within the series as we consider it are two rather different cultural entities The first of these is characterized by thin colonies in which the mycelium is largely submerged and only fruiting structures, commonly arranged in concentric zones, rise above the level of the substratum (fig 59 B) In other strains colonies are rather floccose and somewhat felted and often attain a depth of 2 mm with fruiting structures arising from aerial as well as submerged mycelia Sclerotia generally in purple or black shades regularly and consistently develop in many strains including representatives of both of the above colony types Conidiophores vary greatly in size and characteristically reach their greatest dimensions upon the drier portion of agar slants, or upon slightly moistened grain and other comparable products low in water content The sterigmata in organisms of this group warrant particular attention since the two series commonly but not consistently differ tremendously in size In many strains the primary sterigmata are characteristically wedge shaped and reach dimensions of 25 to 30μ by 10 to 12μ (fig 60 A and C) such structures are commonly septate In other heads from the same culture the primary sterigmata may be relatively small and measure 6 to 8μ by 2 5 to 3 5μ (fig 60 B) Secondary sterigmata are consistently small and of the dimensions indicated in the species description

'White' Aspergilli regularly constitute a normal element in the micro population from moist or improperly dried grains and of comparatively dry vegetation undergoing slow decay From such material many investigators have described molds characterized by white heads which obviously belong in this group but without supplying sufficient critical data to permit subsequent verification of the exact types under study Some of these descriptions were based upon molds growing in culture more of them were not A few of the more tangible of these probable synonyms will be briefly considered in this connection, others will be found in the general species index (pp 331-?)

S alba Bainier (Bull Soc Bot France 27 30 1880) was isolated from oatmeal and while incompletely described obviously represents a member of the *A candidus* series Several publications present elaborate comparative tables to separate strains accepted as *A candidus* and *A albus* but the many strains obtainable vary into each other so completely that little or no basis for separation exists in fact

A albus Wilhelm (Beitr z Kenntn d Pilzgattung Aspergillus Inag Diss Strassburg p 69 1877) was described with characters which clearly ally it with *A candidus* but without sufficient differences to separate it from other members of this group

S blanc jaune Bainier nomen nudum—A culture from Bainier s collection received by Thom under this name (No 4640 490) represents a somewhat diminutive but otherwise typical member of this series

S albo lutea Sartory and Meyer (Cited by Blochwitz in Ann Mycol 31 73 1933) Conidia were reported as turning yellowish in age This character is common to many members of the group and has been so noted by Wehmer (1889-1901) Thom and Church (1926) and others Retention of the species name is not warranted

A basidioseptа Sartory Sartory and Meyer (Ann Mycol 27 317-320 Pl 7 1929) apparently represents a member of the *A candidus* series with comparatively long (28 to 30μ) primary sterigmata which in age are characteristically septate This character which appears also in some members of the *A niger* and *A ochraceus* groups however is not sufficiently unique to warrant specific separation

A niveus var *major* Blochwitz (Ann Mycol 32(1/2) 86 1934) Described as showing vesicles globose rarely oboval or pear shaped entirely covered with radiating sterigmata which are rarely absent toward the base closely growing conidiophores 2 to 2 5 mm high These characters suggest relationship with *A candidus* rather than *A niveus*

A okazakii Okazaki in Centralb f Bakt etc 2 abt 19 p 481-484 taf I 1907 see also Centralb f Bakt etc 2 abt 42 p 225 1914 This is cited by Saccardo in Syll 22 1260 1913 as *S okazakii* Saito but apparently without adequate ground for attributing the name or description to Saito

Colonies described as white to sulphur yellow conidiophores hyaline straight or sinuate smooth or asperulate 200 to 500μ by 8 to 12μ figured as undulate especially toward the base with walls 2 to 3μ thick heads 80 to 100μ in diameter vesicles 12 to 40μ in diameter primary sterigmata 15 to 20μ by 6 to 8μ secondary 8 to 14μ by 2 5 to 4μ conidia globose hyaline 2 5 to 5 4μ smooth with connectives In the event that continued study of these white forms reveals the existence in nature of strains with more or less roughened conidiophores and conidial heads ranging to yellows recognition of *A okazakii* as a separate species would be warranted Based upon current information however we believe it preferable to consider it synonymous with *A candidus* Link

A sachari of Chaudhuri and Sachar (Ann Mycol 32 95 1934) is more or less arbitrarily left where the authors put it—as one of the *A sulphureus* series near *A quercinus* in the *A ochraceus* group The heads are pale yellow the sclerotia are near the colors of that group but the conidiophore is described as colorless and smooth which would put it in *A candidus*

A sterigmatophorus Saccardo in Mycologicae Venetae Specimen Atti d Soc Ven Trent d Sci Nat 2 fasc 2 232 Tab XVII fig 5-8 1873 Syn *S italica* Sacc in F italici no 109 1881 changed to *S italica* Sacc as a note only in Michelia 1 91 1877 Latin diagnosis of *S italica* in Saccardo Sylloge 4 72 1886 Described from decaying corn kernels (*Zea mays*) white sparse with conidiophores unbranched 2 to 3 septate above with vesicles globose sterigmata described as dichotomously or trichotomously branched with ultimate cells bearing conidial chains

conidia globose about 6μ in diameter with connectives The description repea observations as to occurrence and appearance that are frequently seen No o has since reported a member of the A candidus group with conidia 6μ in diamete Whether the organism described by Saccardo was a large spored mutant not an isolated remains open to question

OTHER WHITE ASPERGILLI

Other Aspergilli characterized by white heads but differing basicall in morphology from the *A candidus* group occur in the *Aspergillus glaucu* *A nidulans*, *A fumigatus*, and *A terreus* groups Except for an absenc of spore color these duplicate the morphology of the groups to whic assigned *In fact in all cases except that of A nuveo-glaucus in the A glaucus* group, they represent colorless mutations produced experimentall from typical parent strains (Yuill, 1939, Steinberg and Thom, 1940, Raper Coghill, and Hollaender, in press) While *A niveo-glaucus* was isolate from nature and hence its parentage is not known, it is suspected tha *this represents a mutation of some form close to Aspergillus echinulatus* *A halophilus* of Sartory et al (Ann Mycol 28 (3/4) pp 362–3 Pl 3, 1930) similarly belongs in the *A glaucus* group Attention has been called earlier to the fact that *A candidus* differs from *A niger* primarily in the absence of color and in possessing smooth spores The question may arise whether we are not here dealing with a whole series of mutations from colored forms While this is possible, no proof is at hand The fact that they constitute *such a typical and abundant element of the micropopulation of soil* decaying vegetation, etc , demands that they be considered along with other major groups of the Aspergilli quite aside from any questions of *possible origin*

Sclerotia

In this arrangement of the Aspergilli sclerotia, as compact globose or subglobose bodies composed of thick walled pseudo parenchyma are not *found in the groups characterized by the production of perithecia* and ascospores and only rarely, if at all in groups characterized by the presence of hülle cells *In the great groups beginning with A candidus*, sclerotia appear with sufficient frequency to be morphologically significant as indicative of class relationship Fundamentally the typical *A candidus* strain differs little from the black Aspergilli except for the absence of the dark color and rough spores

Group Relationships

While there is much evidence of relationship with the black Aspergilli (smooth walled conidiophores globose vesicles and heads and the presence of sclerotia) there are also certain *indications of relationship to Aspergillus niveus* Typically both are characterized by snow white conidial

heads and in all strains of *Aspergillus candidus* there are more or less abundant small heads which bear few and loosely arranged sterigmata in a manner strongly suggestive of typical conidial structures of *A. niveus*. Although it is our belief that these similarities in structure do not of necessity reflect close relationship between *A. candidus* and *A. niveus*, we do feel that there is need for additional study of strains which appear to be more or less transitional between the two groups.

Occurrence and Economic Importance

Members of the *Aspergillus candidus* group are very widely distributed in nature and occur with reasonable frequency upon vegetation in the later stages of decay. They are especially common upon moldy grains and are obviously able to grow in the presence of a very limited amount of moisture.

The biochemical and physiological activities of these forms have not been studied extensively. *A. okazakii* was employed by Okazaki (1907 and 1914) for the production of a proteolytic enzyme preparation, digestin, and is the basis of a Japanese patent No. 11461 covering this process. Recently Timonin (1942) has employed a strain reported as belonging to the *A. candidus* group for the production of citrinin. Upon examination, however, this strain is found more nearly to represent *A. niveus* than *A. candidus* in the sense it is considered here.

II Sterigmata in one series (Secondary sterigmata occasional in some strains) *Aspergillus luchuensis* series

A Colonies black or black brown

1 Sterigmata 6 by 3µ (very short) *A luchuensis* Inui

2 Sterigmata about 15 to 20µ conidia 3 to 3 5µ *A nanus* Montagne and/or *A subfuscus* Johan Olsen

B Colonies in reddish brown shades

1 Conidia globose *A japonicus* Saito

2 Conidia elliptical *A violaceo fuscus* Gasperini

Because of their great abundance in nature, the series most closely related to and including van Tieghem's species based upon strains which satisfy his original description in a somewhat broadened sense will be discussed first

ASPERGILLUS NIGER SERIES

Species Characterized by Comparatively Small Primary Sterigmata and Small Conidia

Aspergillus niger van Tieghem, in Ann Sci Nat Bot s 5, t 8 p 240 1867

Synonym *Sterigmatocystis nigra* van Tieghem in Bul Soc Bot France 24 102–103 1877 See also Thom and Currie, Jour Agr Res 7 1–15 1916 and Thom and Church The Aspergilli p 167 1926

Characterization Colonies rapidly growing with abundant submerged mycelium, colorless, or in some strains with more or less yellow color in the hyphae and in the substratum, with aerial hyphae usually scantily produced, but abundant in age in certain strains Conidial heads fuscous blackish brown, purple brown in every shade to carbonaceous black (Pl VI B), varying in intensity with the quantity of coloring matter produced, typically globose or radiate (fig 63 C) commonly up to 300 500, or occasionally 1000µ in diameter with periphery variously splitting into radiating columns of conidia small heads more or less columnar and consisting of a few conidial chains often borne on trailing hyphae or short conidiophores near the substratum Conidiophores mostly rising directly from the substratum uncolored or yellow to brown near the vesicle only smooth with walls thick frequently uneven on the inner surface and splitting lengthwise into strips when broken (fig 64 B and C) unseptate or with occasional thin septa varying greatly in length and diameter in different strains and in colonies on different media or even in sections of the same colony, thus ranging from strains with conidiophores 200 to 400µ by 7 to 10µ to forms with conidiophores several millimeters long and 20µ or more in diameter Vesicles globose or subglobose thick walled com

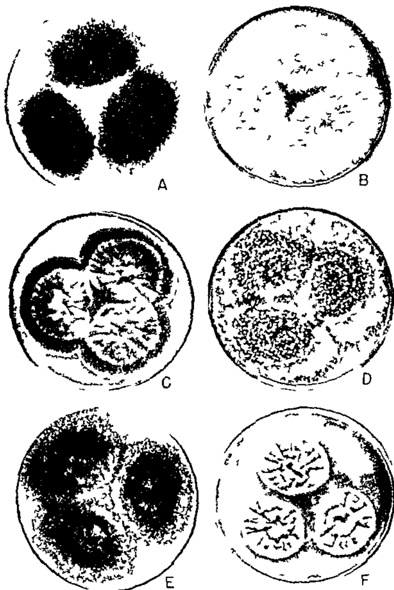


Fig 61 *Aspergillus niger* group cultures growing upon Czapek s solution agar at room temperature 10 days *A A niger* NRRL No 334 typical strain *B A niger* mut *schiemanni* characterized by colonies light brown in color *C A foetidus* NRRL No 341 characterized by a yellowish vegetative mycelium and a strong actinomyces like odor *D A niger* NRRL No 346 characterized by the production of abundant sclerotia *E A phoenicis* NRRL No 1956 characterized by long uncrowded conidiophores *F A violaceo fuscus* NRRL No 360 characterized by compact close textured colonies and small heads with uniseriate sterigmata

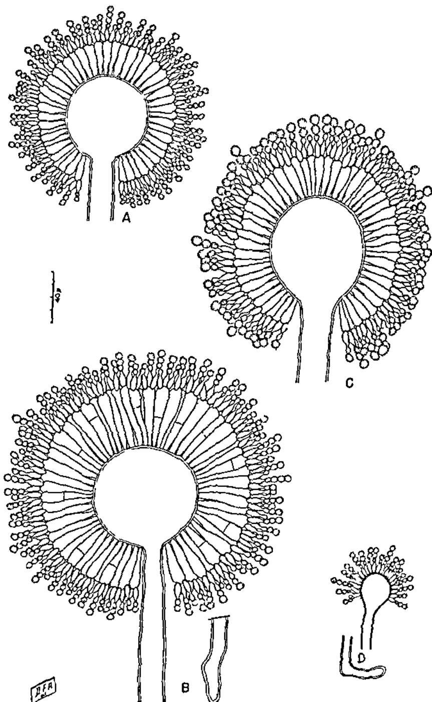


Fig 62 Conidial structures in the *Aspergillus niger* group × 500 *A A niger* NRRL No 326 typical head showing globose vesicle and primary sterigmata about twice the length of secondaries *B A phoenicis* NRRL No 1956 characterized by long and occasionally septate primary sterigmata *C A carbonarius* NRRL No 369 characterized by large primary sterigmata and large conidia *D A violaceo fuscus* NRRL No 360 characterized by small heads and sterigmata in a single series

monly 20 to 50μ occasionally up to 100μ in diameter (fig 62 A) colorless or more commonly more or less intensely yellow brown Sterigmata in one series in young colonies and in small heads but typically in two series colorless at times usually more or less intensely brown even carbonaceous primary sterigmata closely packed covering the vesicle varying greatly in size in the same colony but usually 20 to 30μ in length by 6 to 8μ in diameter at the outer end secondary sterigmata more uniform ranging usually from 6 to 10μ by 2 to 3μ (fig 62 A) both series often more or less brown to almost black Conidia globose when ripe with walls at first smooth with diffused brown or fuscous color (Ridgway Pl XLVI) then rough or spinulose from coloring substance deposited as tubercles bars or loops between the outer primary wall and the inner or econdary wall mostly 2 5 to 4μ occasionally up to 5μ in diameter

Sclerotia globose superficial regularly produced by certain strains (fig 61 D) sporadically by some and not found in many others

A niger approximating the description of van Tieghem furni hes the most common morphological entity among the black Aspergilli A few of the substrata and locations found in our record include chronic irritants in the human ear pin point colonies in the human lung spoiling raw sugar rancid butter and other fats floating and submerged mycelium in many chemical solutions It is abundant in soil cultures from every part of the world and apparently especially so in the tropics Molds under this name have been used in literally hundreds of biochemical investigations

The introduction of a complete description from culture for each member of the series typically represented by van Tieghem s *A niger* but which vary from it in detailed measurements calls for repetition of many common characters In place of such descriptions the names and citations of the forms selected either as unique or as representing sections of the series often encountered are presented with the more important differences which furnish the bases of separation

Aspergillus foetidus n sp

Synonym *A aureus* Nakazawa in Inst Gov t Res Formosa Rept Vol 1 1907
Not *A aureus* Berkeley in English Flora Vol 5 p 346 1836
Not *S aurea* Greco in Origine des Tumeurs et Mycoses Argentines Buenos Aires pp 671–694 fig 418–428 1916

Colonies upon Czapek s solution agar rather slow growing producing a floccose basal mat of mycelium with abundant but uncrowded black heads above a mass of pale orange mycelium which is deeply orange in reverse Heads up to 225μ in diameter are borne on conidiophores about 500μ

long, vesicles comonly 20 to 30μ in diameter, occasionally much larger Primary and secondary sterigmata are both 7 to 10μ by 2 to 4μ conidia are globose spinulose up to 4 or 4 5μ

Colonies have a penetrating actinomyces like odor (also like *Penicillium biforme* noted in Thom, The Penicillia, p 320 1929) unlike any other species of *Aspergillus* Numerous experiments over several years failed to justify the belief that the culture was contaminated with some actino mycete The species is known only from Nakazawa's isolates which have maintained the odor and orange color for many years

In the Awamori fermentation *A foetidus* (*A aureus* Nakazawa) was β, the unfavorable organism which gave a yellow color to the "Koji" used Nakazawa Simo and Watanabe (Jour Agr Chem Soc, Japan, No 144 pp 931–974, illustr, 1936) listed five varieties of *A aureus* as follows var *minor* var *murinus* var *acidus*, var *pallidus* and var *brevis*

Aspergillus awamori Nakazawa in Inst of Govt Res Formosa Rept Vol 1 1907 and Vol 2 1912

The measurements given are only slightly different from those of *A foetidus* (=*A aureus*) The unique odor and the yellow color in the mycelium and substratum were lacking Colonies blackish brown (deep chocolate) when heads were fully developed, conidiophores 1 to 2 5 mm by 9 to 15μ, vesicles globose 30 to 45μ in diameter primary sterigmata 9 to 12μ by 3 5 to 5 5μ and secondary 4 5 to 8μ by 1 5 to 3 5μ conidia globose or somewhat elliptical 3 to 5μ in long axis fairly spinulose

Nakazawa Simo and Watanabe (Jour Agr Chem Soc Japan No 144 pp 931–974 illust 1936) studying the fermentation industries of Formosa found two general types of Aspergilli in the Awamori fermentation, α and β Type α was *A awamori* for which they described the following varieties var *minimus* var *piceus* var *ferrugineus* var *fuscus* var *fumeus* This "awamori" series produced the more desirable type of product citric acid was present as well as alcohol due to the yeast used in the inoculum

Aspergillus niger var *fermentarius* Nakazawa Simo and Watanabe in Jour Agr Chem Soc Japan 10(2) 1934 pp 171–172 summarized on p 181 Reported as a variety with conidiophores 1037 to 2438μ by 13 1 to 16 0μ vesicles 2. 6 to 73 6μ primary sterigmata 12 7 to 16 1μ by 3.3 to 7 2μ secondary 6 8 to 9 8μ by 3 3 to 4 6μ and conidia globose 2 3 to 4 6μ in diameter *This form obviously belongs close to A awamori*

Aspergillus miyakoensis Nakazawa Simo and Watanabe in Agr Chem Soc Japan Jour 12(9) 963–4 fig on 973 1936

The colonies figured by the authors show a cottony mycelium with long stalked heads in a broad zone near the margin The measurements

differ from *A foetidus* as follows primary sterigmata are reported to be 12 to 20μ by 4 4 to 9μ in contrast to secondaries 7 to 10μ by 2 5 to 5μ whereas



FIG 63 Conidia *Aspergillus niger* group *A* Single long chain of conidia × 1000 (Photograph by Edward Yuill) *B* Conidia of a typical strain of *A niger* NRRL No 344 × 700 *C*, Conidia of the citric and gluconic acid producing strain NRRL No 67 × 700 The large size and coarsely roughened walls are characteristic of the conidia of the latter strain

in *A foetidus* both series measure 7 to 10μ by 2 to 4μ conidia are globose 3 7 to 5 6μ in diameter

The species repeats the colony appearance of certain mutants produced by means of chemical stimulants by Thom and Steinberg (1939) from the latter s standard strain of *A niger*

*A henneberg*i Blochwitz, in Ann Mycol 33 238–9 1935

Colonies described as showing the colors and general aspect of *A tamarii* or *A wentii* but with conidiophores browned as in the upper part of the conidiophores of the *A niger* group and with red sclerotia relationship doubtful See also the *A wentii* group

Aspergillus niger van Tieghem, in Ann Sci Nat Bot s 5 t 8 p 240 1867

Van Tieghem's strain is not fully verifiable among organisms now maintained in culture, although Biourge (personal communication) believed he had it We believe the name can be most appropriately used to cover

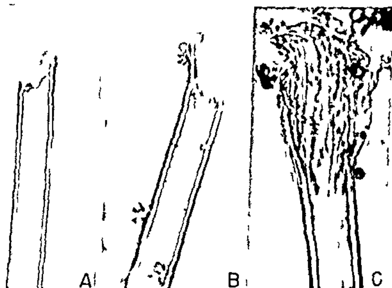


Fig 64 Broken conidiophores of *Aspergillus niger* NRRL No 3?6 × 700 *A* Conidiophore showing a clean break suggesting a glass tube *B* Broken conidiophore showing fibrous wall structure *C* Portion of conidiophore crushed and further revealing the fibrous structure of the wall

the exceedingly abundant isolates having the approximate measurements of sterigmata and conidia noted in van Tieghem's description The reader can assume therefore that in the opinion of the authors any possible description for the species itself would read essentially like that already given for the series on pages 216–219

Species Characterized by Large Primary Sterigmata and Small Conidia

A phoenicis (Corda) Thom in The Aspergilli p 175 1926 In describing the black Aspergillus found upon dates Patouillard and Delacroix (Bul Soc Myc France 7 118–120 1891) compared their material to specimens

in the museum labeled *Ustilago phoenicis* and attributed to Corda, thus establishing the identity of the organism of Corda which was not recognizable from any previous references *A ustilago* Beck (1892) is described with the same measurements The measurements of sterigmata place *A phoenicis* in the section of the group with primary sterigmata 50 to 60μ in length it was first described as *Ustilago phoenicis* by Corda (Icones Fung IV p 9 pl 3 fig 26 1840) and transferred by Patouillard and Delacroix as noted above to *S phoenicis* (Corda) Patouill and Delacr If we accept the use of phoenicis attributed to Corda as correct this species becomes the type of the section of the black Aspergilli with primary sterigmata of intermediate length and conidia not over 4μ in diameter (fig 61 E and 62 B) This was cited by Thom and Currie as *A phoenicis* (Corda) Pat and Delacr and continued recognition of the species to cover a group of strains occasionally encountered appears warranted

Aspergillus pulverulentus (McAlpine) Thom in Jour Agr Res 7 10–11 1916

Synonym *S pulverulenta* McAlpine in Agr Gaz N S Wales (1896) 7 302 1897 See also Thom and Church The Aspergilli p 179 1926

McAlpine s data include White to dirty yellow mycelium heads 155 to 340μ in diameter radiate with chains mostly separate conidiophores erect stiff up to 7 mm by 20μ with walls up to 5μ thick vesicles 70 to 170μ in diameter globose or nearly so primary sterigmata up to 144μ long by 8μ secondary 14 to 18μ long conidia globose rough about 4μ in diameter Colonies with these general characters have been studied in culture at least twice and maintained for long periods one came from Spain the other from Texas *A strychni* Lindau (Hedwigia Bd 43 Rept 5 p 306–7 1904) is one of this series

Light Colored Forms

Aspergillus niger mut *cinnamomeus* (Schiemann) n comb

Synonym *A cinnamomeus* Schiem in Ztschr Induktive Abstam u Vererbungslehre Bd 8 Heft 112 pp 1–35 16 fig 2 pl (1 col) 1912 See also Thom and Church The Aspergilli p 164 1926

Colonies upon Czapek s solution agar at room temperature rapidly growing and spreading producing an aerial growth of conidiophores and heads reaching a pale cinnamon upon maturity Reverse only slightly

colored in the same shade Conidial heads not crowded globose Conidiophores smooth, thick walled, with upper portion more or less brown, about 1 5 mm in length by 12 to 20μ in diameter Vesicles up to 40 to 50 μ in diameter, crushing readily Sterigmata in two series primary about 15 to 20μ by 3 to 5μ, sometimes larger, secondary about 8 by 2 to 3μ Conidia 3 to 4μ, thin walled globose or subglobose smooth or nearly so almost colorless when viewed singly, pale yellowish to cinnamon in mass

Diagnosis based upon culture NRRL No 348 (Thom No 3534b) received from Schiemann as a mutation induced by introducing potassium bichromate into the culture medium Approximately the same mutant appeared in Steinberg and Thom's series of induced mutations (1939, 1940) Occasional cultures close to *A cinnamomeus* have ben obtained from unknown sources in nature

Aspergillus niger mut *Schiemanni* (Schiemann) n comb

Synonyms *A Schiemanni* (Schiemann) Thom, Jour Agr Res 7 13 1916

A fuscus Schiemann, in Ztschr Induktive Abstam u Vererbungslehre Bd 8, Heft ½, p 1-35, 10 fig 2 pl (1 col) 1912

Not *A fuscus* Bonorden (Bot Ztg Jahr 19 202 1861),

Not *S fusca* Bainier (Bul Soc Bot France 27 29, Pl 1, fig 5 1880)

Colonies upon Czapek's solution agar at room temperature rapidly growing and spreading, developing a surface growth of conidiophores and heads forming a crowded fruiting area 2 to 3 mm deep in slanted tubes, becoming a shade of brown near fawn color (Ridgway, Pl XL) reverse yellowish (fig 61 D) Conidial heads large, fairly crowded Conidiophores coarse, 2 5 mm or more long by 15 to 25μ wide Vesicles up to 50 to 60μ in diameter Sterigmata in two series, primary 15 to 40μ by 4 to 6μ, sometimes larger secondary 7 to 8μ by 2 to 3μ Conidia thin walled smooth except sometimes a trace of markings 3 5 to 4 5 or 5μ in diameter

Culture NRRL No 361 (Thom No 3534C) was received from Schiemann Culture NRRL No 362 was received from Biourge under the same name but shows a much deeper brown color (near Natal brown Ridgway Pl XL)

The mutant *A niger* mut *Schiemanni* is distinguished from the parent type of *A niger* by the color and smoothness of its spores The name, *A fuscus* Schiemann is invalidated by the prior usage of the names *A fuscus* by Bonorden and *S fusca* by Bainier

Mutations of approximately this same type have been secured from cultures of strain NRRL No 67 (Pl VI C) by ultra violet irradiation

(Raper Coghill and Hollaender), from cultures of other black Aspergilli through Cathode ray irradiation (Whelden 1939) by cultivation in the presence of various chemicals (Steinberg and Thom 1939 1940) and finally by the appearance in plate culture under normal conditions of a light-spored sector in an apparently typical culture of *Aspergillus niger* See discussion Chapter VI Two isolates labeled *A awamori* Nakazawa which resemble this mutant in color have been received from Japan one from Hanzawa the other from Nakazawa Both produce primary sterigmata less than 20μ in length but otherwise are close to Schiemann s mutant

Probable Synonyms

Many species have been described by investigators working at different periods and at widely separated stations which obviously belong within the *Aspergillus niger* series as it is here considered Some of these will be briefly noted since they were reported to present unique cultural or morphological features or since they account for interesting or important biochemical reactions

A giganteus Mattlet (Ann Soc Belge Med Trop 6 36 1926) was described as a new species with conidiophores 2 to 6 mm long conidia 4 to 5μ in diameter rough black brown but without other marks of separation It would seem to have been a rather extreme variant in the two characters

A atropurpureus Blochwitz (Ann Mycol 32(1/2) 86 1934) Not *A atropurpureus* Zimmermann (Centralb f Bakt etc 2 Abt 8 No 5/7 p 218 1902) Blochwitz proposed to use the name *A atropurpureus* for the purple brown members of the *A niger* group as he obtained them from the tropics He gave conidial measurements as 2 5 to 3 5μ in diameter and added that chemical differences in the coloring substances warranted separation from the black or blacker forms

A ficuum (Reich) Hennings in Hedwigia 34 p 86 1895 and Reichardt in Verhandl K K Zoll Bot Gesell Wien 17 335 1867 Regarded as *A niger* by Wehmer in Centralb f Bakt etc 2 Abt 18 No 13/15 p 394–395 1907 See also Thom and Church The Aspergilli p 174 1926

Slight differences in morphology between this and *A niger* v Tieg are reported by Hennings but disregarded by Wehmer Culture NRRL No 364 (Thom No 142) received in 1909 from Westerdijk under this name was reported by Thom and Currie (1916) as the most rapid producer of oxalic acid of all strains of *A niger* tested There is no morphological basis for separating this strain from *A niger* and it is distinguished in culture only by the tendency after many years in laboratory culture to produce rather floccose colonies

A batatae Saito in Centralb f Bakt etc 2 Abt 18 No 1/3 p 34 1907 Saito s organism as described is close to *A niger* colonies reach brownish black through yellow shades conidiophores 2 to 4 mm by 12 to 20μ smooth thick walled brown in upper portion vesicles 35 to 50μ globose primary sterigmata 24 to 40μ long by 8μ at apex secondary 10 by 3 [illegible]μ conidia globose brown finely roughened 4 to 5μ in diameter A culture under this name from Formosa (NRRL No 363) presented the

comparatively large conidia described by Saito but possessed no other marks which would assist in identification

A luteo niger (Lutz) Thom and Church in The Aspergilli p 166 1926 Syn *S luteo nigra* Lutz in Bull Soc Bot France 53 48–5? 1907 Thom and Church (1926) found one or more black Aspergilli in which the conidia appeared smooth in ordinary laboratory slide amounts in which they were treated with alcohol followed by lacto phenol or other mounting fluids Fragments floating in the mounting medium led to the test of such conidia in dry mounts in oil and in pure glycerine Under these conditions the conidia showed the typical marks of the *A niger* series Since smooth conidia were the sole definite contrast between *A luteo niger* Lutz and *A niger* v Tiegh the strains studied were accepted as Lutz organism Since that time, it has been shown that many black Aspergilli ranging widely among the variant forms encountered pass through a physiological stage in which the outer wall and color bars when subjected to alcohol or other fluid causing active osmotic currents break in pieces and float away leaving the conidia smooth and colorless or only partly shaded toward the dark color of the group It thus appears that *A luteo niger* may be dropped as failing to designate any definite group of strains and as failing to present any character of diagnostic importance

ASPERGILLUS CARBONARIUS SERIES

Species Characterized by Conidia in Excess of 5.0µ—Sterigmata in Two Series

Aspergillus atropurpureus Zimmermann, in Centralb f Bakt, etc, 2 Abt, 8, No 5/7, p 218 1902

Conidiophores hyaline or somewhat brownish in age up to 800 by 16µ to 20µ, vesicles 60 to 80µ in diameter, hyaline to brown sterigmata primary 16 by 6µ secondary 3 to 4µ by 1 5 to 2 0µ conidia globose rough with prominent warts, purplish black, 6 to 10µ in diameter Isolated from *Coffea liberica*, in Java Culture not studied by us The species is possibly valid and is presented as representative of occasional forms characterized by small sterigmata and large purple black spores

Aspergillus fumaricus Wehmer in Ber Deut Chem Gesell 51 No 14 1663–1668 figs 1–6 1918

This species was named but not fully described by Wehmer and was not distributed by him Its biochemical activity as a producer of fumaric acid is covered in the paper cited above together with the admission that it belonged to the *A niger* group Culture No 4668 2 (C Thom) received from Neuberg under this name presents a variant strain of the group which may be described as follows Colonies producing a mass of yellow mycelium, conidiophores scantily and tardily produced up to 1 2 or 3 mm long, by 20 to 22µ in diameter smooth, bearing large radiate yellow brown heads Vesicles up to 60 or even 100µ in diameter with walls thin easily crushed Sterigmata in two series primary sterigmata up to 15µ by 4 to

5µ secondary sterigmata rather coarse some growing out into aborted hyphae Conidia commonly 5µ occasionally 7 to 8µ in diameter with lengthwise color bars as in A niger but paler in color

Culture No 4668 2 (Thom) is accepted as correctly named By description, *A fumaricus* Wehmer differs little from *A atropurpureus* Zimm except for the production of yellow brown rather than purple black conidial heads Differences in color definition and discrimination by different workers tend to leave both species in doubt

Aspergillus fonsecaeus n sp

Synonym *S fusca* Bainier in Bul Soc Bot France 27 29 Pl 1 fig 5 1880 (Bainier s material grown upon moist bread in Toulouse in 1880 was preserved in Roumeguère s Fungi Gallici Exsiccati No 995)

Bainier described *S fusca* in terms closely parallel to van Tieghem's *A niger*, except that the conidia were about double that species in size Bainier reported these as rarely exceeding 9 4µ in diameter, while our examination of the exsiccati showed them to be mostly 5 to 8 5µ in diameter and marked as in *A niger* and *A carbonarius* Da Fonseca in Rio de Janeiro contributed a culture (Thom No 4707 878, NRRL No 67 and strain No 67 of Herrick May, and associates) possessing approximately these spore measurements which has retained its characteristic features for more than 20 years and which has proved industrially useful We believe therefore that recognition of a species with sterigmata of intermediate length and conidia about double the dimensions of *A niger* van Tieghem is warranted The name *A fonsecaeus* is proposed since the binomial *A fuscus* had already been used for an Aspergillus by Bonorden in 1861 (Bot Ztg Jahrg 19 No 29 p 202) and has been used at least twice subsequent to this The species would then include strains characterized by large subglobose coarsely roughened conidia ranging from 5 5 or 6 0µ to 8 5 or 9 0µ (fig 63 C) including the strain 67 used by Herrick May Wells Moyer et al of the Industrial Farm Products Research Division U S Department of Agriculture Arlington Farm Virginia for the production of citric and gluconic acids (see literature citations pp 290–295)

The following description is based primarily upon strain NRRL No 67

Colonies upon Czapek s solution agar growing rapidly at temperatures from 24 to 30 C attaining a diameter of 7 to 8 cm in eight to ten days consisting of a basal vegetative mycelium that is largely submerged and colorless and abundant conidial structures commonly arranged in more or less conspicuous concentric zones heads carbon black or brownish black imparting to the colony a like coloration reverse colorless in young colonies

commonly darkening in age and often becoming *almost black after 2 to 3 weeks* Conidial heads large, globose radiate or with chains of conidia massed in an indefinite number of loose divergent columns, commonly 300 to 500μ but often attaining a diameter up to 1 mm Conidiophores varying in length from 1 5 to 3 5 mm but averaging about 2 0 to 3 0 mm, mostly 20 to 30μ in diameter with walls 2 0 to 3 0μ thick smooth, often colored in dark shades in the region beneath the vesicle Vesicles globose, fertile over the entire surface commonly 50 to 75μ in diameter (fig 62 C), usually *in brown shades, often quite dark* Sterigmata in two series, usually brown, often dark primary sterigmata variable in different heads and in different cultures ranging from 15 to 20μ by 6 to 8μ in some to 35 to 45μ by 10 to 13μ in others secondary sterigmata ranging from 8 to 14μ by 5 to 6 5μ but averaging about 9 to 10μ by 5 to 6μ Conidia large (fig 63 C) *globose conspicuously roughened with prominent color bars ranging from* 5 5 to 8 5μ

Since strain "67" appears in the industrial fermentation literature as *Aspergillus niger* and has been consistently distributed under this name over a period of several years, it is not our purpose here to challenge this designation, for this binomial *is often used in a very general sense to cover* any black member of this group We do wish to emphasize however that this strain does not represent the common type of black Aspergillus usually isolated in routine examination of soil and moldy materials in general The fact that it possesses large spores is of the greatest value in checking its purity and further commends it for use in industrial operations This strain was originally received from Rio de Janeiro and recently Dr Dorival M Cardoso of Sao Paulo Brazil has reported (personal communication) *that he has repeatedly isolated large spored forms apparently closely* related to it It would, therefore, appear likely that this large spored form represents a type of organism much more abundant in South America than in this country

Aspergillus pulchellus (Speg) Thom and Church in The Aspergilli p *181* 1926

Synonym *Aspergillopsis pulchellus* Speggazzini in Myc Arg V in Ann Mus Nac Buenos Aires Ser *3* t *13* *436* *1911*

This species was described with colonies intensely black conidiophores 1 to mm by 18 to 20μ with walls darkened vesicles 50 to 60μ in diameter primary sterigmata 10 by 30μ and conidia 8 to 10μ in diameter and rough The species appears to differ from van Tieghem s *A niger* principally in its very large conidia and it is extremely doubtful if it could be separated from *A fonsecaeus* as described above

A dipus of Ferdinandsen and Wing (Bot Tids *30* *220* fig *6* *1910*) *represents* another organism undoubtedly close to the forms under consideration The presence of conspicuous foot cells upon which character the species was based is common to all members of the group hence *is not a valid basis for separation*

Aspergillus carbonarius (Bainier) Thom in Jour Agr Res 7 12 1916

Synonym *S carbonaria* Bainier in Bul Soc Bot France 27 27–28 1880

Colonies grown upon Czapek s solution agar show vegetative mycelium white or with some yellow in submerged areas broadly spreading more or less zonate sclerotia produced upon the surface of the substratum in old cultures fruiting areas carbon black Conidiophores colorless below yellow to yellow brown toward the apex 4 to 6 mm or more in length and up to 25μ in diameter with walls smooth sometimes as much as 4μ in thickness Heads globose varying in diameter up to 500μ Vesicles up to 90μ in diameter fertile over the entire surface commonly with contents yellow brown to black and in old heads forming with the primary sterigmata a hard brittle carbonaceous mass Sterigmata in two series primary sometimes one septate from 20 to 40μ long in young or small heads and up to 120μ long in large heads by 5 to 13μ in diameter at the apex secondary 8 to 14μ by 3 to 6μ Conidia at first smooth becoming rough when ripe 5 5 to 10 5μ in diameter Colonies grow well upon all culture media used with temperature optimum below 37 C A culture from Dr A F Blakeslee (NRRL No 369 Thom No 4030 1) reproduces in detail the morphology recorded by Bainier *A carbonarius* has also been received in culture from the Gold Coast of Africa

The same morphology was also found in one of Dr Farlow s specimens *S acini uvae* Caballero (Bol R Soc Esp Hist Nat 28 429 1928) This was described as it appeared upon rotting grapes as follows vesicles 75 to 105μ by 73 to 98μ primary sterigmata 59 to 80μ by 13 to 22μ and secondary 15 to 20μ by 5 to 7μ conidia globose rough 6 to 10μ Blochwitz in Die Aspergillaceen (Ann Mycol 27(3/4) 204 222 and 232 1929) with part of the type specimen before him declares it to be *A carbonarius* apparently without cultivating it Mosseray (LaCellule 43 222 1934) places Caballero s organism in *Aspergillus pulchellus*

We cannot agree with Blochwitz (idem p 221 222 fig 12) in describing the vesicle in *A carbonarius* as subglobose and figuring the head as hemispherical Bainier s original figures show the vesicle fertile to the very base This is characteristic of the strains observed from various sources

ASPERGILLUS LUCHUENSIS SERIES

Species Normally Showing One Series of Sterigmata Occasionally Two

Aspergillus luchuensis Inui in Jour Col Sci Imp Univ Tokyo 15 469 Pl 22 fig 1–8 1901 See also Usami in Centralb Bakt etc 2 Abt 43 p 250 1915 The Aspergilli p 171 Pl IV 1926

Colonies upon Czapek s solution agar spreading rapidly producing abundant conidiophores and conidial heads which give a purple black color

to the whole colony, reverse in pale yellow shades Conidial heads globose, up to 250 to 300μ in diameter, splitting in age into short columns of spores Conidiophores up to 1500μ by 10μ smooth yellow toward the vesicle Vesicles yellow, up to 40μ in diameter fertile over the entire surface Sterigmata mostly in one series 6μ or a little larger by 3μ, branched sterigmata occasionally appear Conidia globose, 3 5 to 4μ roughened with spines rather than bars of coloring substances

Representatives of the species have been received from Japan (NRRL No 356, Thom No 4291 3) from West Africa from Bermuda, and from various points in the United States While not as abundant as forms with double sterigmata isolates with this kind of head are not uncommon

Inui also described *A perniciosus* (Jour Coll Sci Tokyo 15 p 473 T XXI, figs 9–12 1901) with color data closer to *A wentii* than *A luchuensis* Its morphology however seems to belong here It has not been rediscovered and discussed adequately in relation to either group *Culture No 4707 757* (Thom), received from da Fonseca in Brazil, possibly represents this species colonies in center at least transiently greenish but without true green color conidiophores not crowded sinuous apparently smooth when observed with low magnifications but with traces of pitting evident when e m ied with an oil immersion objective up to 1000μ by *10 to 15μ* primary sterigmata 10 to 16μ by 3 to 4μ secondary up to 8μ by *2 to 3μ*, conidia 4 to 5μ in diameter with yellow markings in the form of loops and bars

A luchuensis var *rubeolus* Shih (Lingnan Sci Jour 15(3) 374 1933) differs from the species by becoming chocolate brown rather than black This would suggest careful comparison with *A japonicus* Saito

Aspergillus japonicus Saito in Bot Mag (Tokyo) 20 61 5 figs 1906

Colonies upon Czapek's solution agar growing rapidly and spreading evenly characterized by its purple brown heads and the presence of few to many light brown sclerotia reverse colorless or nearly so Conidiophores 500 to 1000μ by 12 to 15μ figured as showing concretions on the surface with walls more or less brown Vesicles globose fertile over the whole surface with walls brown and marked by the bases of sterigmata Sterigmata in one series 7 to 9μ by 5 to 6μ commonly falling away in mounts from old cultures Conidia globose echinulate 4 to 5μ in diameter Sclerotia scattered throughout the colony 650 to 1000μ in diameter white to pale yellow in color and often overgrown by mycelium and conidiophores

Type material has not been seen The diagnosis is based upon two strains contributed by Dr A F Blakeslee (NRRL No 358 Thom No 4030 3 and NRRL No 359 Thom No 4030 5) which come fairly close to

Saito's description A purple brown strain (Thom No 5362 7) collected by Manns in Honduras showed lightly darker colors and slightly different measurements A strain received from Raistrick as coming from Blochwitz through the Centralbureau in Baarn labeled *A atropurpureus* Zimmermann resembled the two Blakeslee cultures quite closely It certainly did not comply with Zimmermann's description (see p 226) *A luchuensis* var *rubeolis* Shih is probably closely related if not identical with *A japonicus* Saito

Aspergillus violaceo fuscus Gasperini in Atti Soc Toscana Sci Nat Pisa Mem 8 fasc 2 p 326 1887

Colonies upon Czapek's solution agar comparatively slow growing (fig 61 F) purplish brown with a faint violet shade passing to purple drab in age reverse colorless to dark purplish Conidial heads purplish brown, globose not crowded 100 to 150μ in diameter Conidiophores mostly less than 1 mm in length but sometimes reaching 2 mm 12 to 18μ in diameter Vesicles globose varying up to 60μ in diameter Sterigmata generally in one series (fig 62 D) 5 to 8μ by 3μ occasionally in two series with secondaries 2 to 4μ long Conidia elliptical 3 5 to 5μ by 5 to 6 5μ at first hyaline becoming violaceous somewhat roughened

By description this is a variant member of the great group of black Aspergilli characterized by short sterigmata and elliptical conidia

Gasperini's material has not been seen but three cultures have been studied which are believed to differ from his species only in having somewhat smaller conidia The first of these was received in 1914 from Puerto Rico (NRRL No 360 Thom No 3522 30) while the others were subsequently obtained from Jamaica and from Professor Raistrick in England

In cultivating black Aspergilli an occasional strain produces heads at first showing a single series of sterigmata then as the colony becomes older heads with both primary and secondary sterigmata dominate the culture Upon careful examination many strains show both large heads with sterigmata in two series and on shorter conidiophores mixed among the larger ones small heads with simple sterigmata only or with both types mixed Colonies of this kind probably accounted for *A nanus* Montagne *A subfuscus* Johan Olsen (Sopp) and are known to account for *A pyri* English

Aspergillus nanus Montagne in Syllog Generum Specierumque Cryptogamarum p 300 No 112 Paris 1856 Sacc Syll 4 71 1886 Species reported as a member of the black group with a single series of sterigmata about 15μ in length and spores 3μ in diameter This may have represented young fruiting structures of a typical *A niger*

Aspergillus subfuscus Johan Olsen in Meddelelser fra Naturh forening i Kris

tiania 1885 Described as showing smooth globose spores 3 0 to 3 5μ and a single series of sterigmata in culture up to 20μ in length but with some secondary sterigmata seen in the original material The separation of this from *A niger* appears questionable

Aspergillus pyri English n n in Doctoral Thesis State College of Washington Pullman Wash pp 76-78 1940 cited in abstract A form described as showing a single series of sterigmata 14 4 to 19 2μ by 3 6μ and spinulose conidia 3 6 to 4 8μ in diameter In personal correspondence of June 1943 English stated that as a result of continued study of this strain and the finding of double sterigmata he questioned the desirability of maintaining the species designation

SYNOPSIS OF SPECIES PROPOSED BY MOSSERAY

Biourge, who was a discriminating collector, accumulated 63 strains of "black (or related) Aspergilli for each of which he could see sufficient individuality to warrant preservation Mosseray, working in Biourge's laboratory, studied these strains, attempted to establish species lines among them, and proposed new specific names for all forms which he believed undescribed With the whole 63 strains in parallel culture, all bearing the names applied by Mosseray, Biourge and Simonart were inclined to withdraw part of Mosseray's new species names a position with which the writers heartily agree Nevertheless to present one concept of the range of variation confronted by the student of this group Mosseray s synopsis[1] has been translated with minor emendations, and is herewith presented

Mosseray s Synopsis

A Conidia 6 to 10μ in diameter rough vesicles subglobose primary sterigmata often 100μ or more in length colonies jet black

a Sporulation more or less dense heads large reverse fumose or very dark olive with mycelium more or less wrinkled

Conidiophores 2 4 or even 6 mm long sclerotia present in ' natural media *A carbonarius* (Bainier) Thom and Currie

Conidiophores 1 to 2 mm long sclerotia not reported *A pulchellus* (Speg) Thom and Church Syn *S acini uvae* Caballero

b Sporulation less dense heads small also with very small heads with single

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[1] Translated and emended from Mosseray Raoul Les Aspergillus de la section niger Thom and Church in La Cellule XLIII 271-273 1934 All data are based upon colonies grown in slanted test tubes using Biourge s formula of neutral Raulin agar of the following composition

Water (distilled)	1000 cc	$(NH_4)_2HPO_4$	0 400 g
Sucrose	50 g	$(NH_4)_2SO_4$	0 700 g
Tartaric acid	0 40 g	$FeSO_4$ cryst	0 050 g
$MgCO_3$	0 250 g	$ZnSO_4$	0 050 g
NH_4NO_3	2 500 g	Agar	20 0 g
K_2CO_3	0 400 g		

Sterilized at 120 C for 20 minutes

sterigmata and on very short conidiophores among them mycelium gray yellow reverse dark reddish brown and mycelium much wrinkled
A pseudo-carbonarius (Bainier nn) Mosseray

B Conidia 2.5–4 even to 5μ in diameter mostly more or less rough and more or less colored but some smooth or nearly so vesicles normally globose primary sterigmata mostly 20μ long or longer sometimes up to 100μ

a Colonies deep purple brown or clear purple brown

I Conidiophores short (up to 2 mm on an average)

α Wrinkles in reverse shallow transverse (in tubes)

Heads small conidiophores short (up to 1 mm) 8 to 15μ in diameter primary sterigmata 5 to 20μ reverse cream colored
A microcephalus Mosseray

Heads medium conidiophores up to 2.5 mm primary sterigmata up to 45μ reverse dark reddish brown often spotted and becoming very dark. *A phoenicis* (Corda) Thom
Syn *A longobasidia* (Bainier n n) Mosseray
Syn *A bainieri* Mosseray 1934

β Wrinkles in reverse of mycelium fairly numerous occasionally anastomosing

Appearance mealy deep purple brown conidiophores very short up to 0.5 mm and 7 to 13μ in diameter primary sterigmata 4 to 12μ reverse deep olive brown *A pseudo-citricus* Mosseray

Appearance sub granular brown purple at times clear brown to umber conidiophores up to 1.5 mm by 7 to 20μ conidia smooth or nearly so growth rapid Reverse slightly colored

Reverse cream to pale brown primary sterigmata 8 to 20μ
A fuliginosus Peck
Syn *S fuliginosa* Bainier
Syn *A praecox* Mosseray 1934a

Reverse very deep brown primary sterigmata up to 50μ sclerotia occasional *A sclerotifer* Mosseray

Reverse uncolored to citron yellow primary sterigmata 8 to 20μ
A citrino niger Mosseray

γ Wrinkles sharp and numerous (reticulated) Reverse becoming rapidly dark almost black

Aspect mealy color purple brown conidiophores up to 1 mm drops not colored primary sterigmata 12 to 30μ conidia smooth or nearly so *A densus* Mosseray

Aspect mealy to granulate deep purple brown drops numerous bronze primary sterigmata 12 to 30μ *A rutilans* Mosseray

Aspect subgranular brown purple drops numerous black some of them very large primary sterigmata 1? to 30μ
A guttifer Mosseray

Reverse not so dark sometimes olive or colorless

Reverse deep olive often spotted primary sterigmata 15 to 30μ
A Buntingii Mosseray

Reverse not colored commonly showing areas sterile or free from spores mycelium slightly yellow at first primary sterigmata 20 to 50μ *A variegatus* Mosseray

II Conidiophores up to 3 mm rarely 4 mm long

α Sporulation normal

Reverse uncolored or slightly olive drops few or none primary sterigmata 20 to 40μ mycelium often yellow at first *A niger* Van Tieghem

Reverse orange brown or purple brown drops large black primary sterigmata 8 to 25μ mycelium colorless or rarely yellow *A Bourgei* Mosseray

Reverse pale reddish brown drops very few heads very small primary sterigmata 15 to 30μ mycelium colorless or sometimes rose sporulation very slow *A Churchii* Mosseray

Reverse golden yellow, drops none primary sterigmata 20 to 40μ mycelium reddish yellow at first *A luteo niger* (Lutz) Thom and Church

Reverse dark olive brown conidiophores up to 1 to 2 mm primary sterigmata very variable vesicles subglobose *A anomalus* Mosseray

Reverse cream to brown spotted slightly wrinkled with a dark band in center sporulation scattered pale conidiophores up to 4 mm long *A tubingensis* (Schober) Mosseray

β Sporulation abnormal

Sporulation absent in patches and in the margin with a granular appearance and proliferation of mycelium throughout the conidial area *A granulatus* Mosseray

Sporulation massed at the thin end of the agar less dense toward the bottom of the tube mycelium wooly

Mycelium wooly gray white or yellowish gray carrying on the thin areas numerous simple heads (fumigatiformes) and some normal heads Reverse cream slightly wrinkled *A velutinus* Mosseray

Mycelium less wooly white conidiophores 1 to 3 mm long very abundant at the thin end of the agar the remainder sterile Reverse much wrinkled cream *A ficuum* (Reich) Hennings

III Conidiophores tall up to 1 cm sporulation scanty mostly toward the thin end of the agar

Heads large splitting into divergent columns conidiophores up to 1 cm by 30μ primary sterigmata up to 100μ reverse cream or slightly rose *A elatior* Mosseray

Heads small mycelium gray rose conidiophores rarely up to 1 cm more often 2 to 6 mm very numerous little fumigatiform heads on short conidiophores at the surface reverse clear rose or salmon *A pseudo elatior* Mosseray

IV Conidiophores very irregular from less than 1 mm to 3 mm with much larger heads reverse clear reddish brown mycelium yellow at first then dark brownish red sporulation delayed *A pseudo niger* Mosseray

b Colonies not purple brown but clear brown morphology of *A niger* conidia mostly smooth or nearly so

I Colonies clear brown or sepia conidiophores up to 1 mm reverse olive or lighter with reticulated wrinkles vesicles 20 to 40μ primary sterigmata 12 to 50μ conidia smooth *A olivaceo fuscus* Mosseray

II Colonies umber conidiophores 1 to 3 mm reverse reticulated dark reddish brown conidia smooth or nearly so *A Schiemanni* Thom
Syn *A fuscus* Schiemann

III Colonies reddish salmon conidiophores 1 ? or even 3 mm reverse slightly wrinkled uncolored or pale cream conidia smooth
A cinnamomeus Schiemann

C Conidia 3 to 5μ globose smooth slightly colored vesicles globose conidiophores up to 1 mm slender primary sterigmata 12 to 20μ colonies appearing somewhat granular deep brown reverse olive passing to bronze mycelium sulphur yellow at first *A citricus* (Wehmer) Mosseray

D Conidia 3 to 5μ globose or elliptical smooth or slightly rough colonies violaceous

a Sterigmata in two series vesicles globose colonies purplish violet or mauve reverse violet brown conidia 3 to 5μ globose smooth uncolored
A awamori Usami

b Sterigmata in one series short vesicles subglobose colonies in violaceous shades to mauve

Colonies mauve (violet livide) reverse uncolored wrinkled conidia globose or obovate smooth 3 to 5μ in long axis sporulation slow narrowly growing *A malvaceus* Mosseray

Colonies violaceous or dark violet slate reverse dark yellow or orange slightly wrinkled conidia globose and rough 3 to 4 5μ sporulation very rapid and colonies broadly spreading with sclerotia common on rice or other natural substrata rare upon sugar media *A japonicus* Saito

Colonies violet brown reverse purplish brown wrinkled conidia globose rough 3 to 5μ in long axis sporulation slow and more or less incompletely covering the surface dwarf heads abundant *A atro violaceus* Mosseray

Colonies dark brown or carob brown with a mealy or granular appearance reverse dark brown or olive at the margins wrinkled conidia smooth globose 2 5 to 4μ vesicles globose or pyriform primary sterigmata 3 5 to 7 5μ in diameter *A atro fuscus* Mosseray

If the material available for study were limited to Biourge s 63 cultures identification to strain variety or species might be possible When Mosseray subsequently returned to Brussels as Mycologist at the Jardin Botanique de l Etat and was confronted by hundreds of other strains largely from the Belgian Congo many of which presented further variation he began to see the impossibility of describing them all in terms which would permit subsequent identification Biourge at that point (personal conference) withdrew his support from many of the diagnostic features accepted in Mosseray s mémoire and agreed to the proposal that specific names in black Aspergilli could only be serviceable as bringing together aggregates of strains showing common and fairly dependable morphological characters This was the attitude held by Thom and Church in The Aspergilli (1926) based upon the examination of many hundreds of black Aspergilli and it remains the position of the writers at this time

Since variation is characteristic of the whole genus—not one group

alone, the subject is covered in the chapters on Morphology and on Variation

Coloration

Color has been emphasized in most of our attempts to separate these strains in culture Linossier (1891) extracted from his strain of *A niger* a coloring substance soluble in hot water which he called aspergilline This substance is readily demonstrated Blochwitz (1929, p 219) reports this material to be soluble in "NH_3 and alkalies" and we have extracted it with hot water Microscopic comparison of conidiophores heads, and separate conidia from numerous strains leads to the conclusion that the same substance may give a brown color to the upper one third of the conidiophore, to the vesicle and its contents, to the walls of the sterigmata, and in *A carbonarius* so fill the whole vesicle and sterigmatic area with a brittle mass as to justify Bainier in calling the head carbonaceous Conidia in a few strains have appeared to possess smooth, uniformly brown cell walls As a group however the black aspergilli have globose conidia with a firm, uncolored or slightly colored inner wall a very thin outer wall and between the two the coloring substance presumably aspergilline deposited as granules warts or bars running lengthwise of the cells and presenting a pattern characteristic of the whole group When pale colored variants (mutants?) such as *A cinnamomeus* (p 223) or darker ones such as *A schiemanni* (p 224) or the variously brown to deep carbon black ones like *A carbonarius* are compared the relative quantities of coloring matter seem to account for the progressive darkening of the head colors as described in the series

In the 'reverse or underside of the mycelium and in the culture substratum, a range of shades from colorless to yellows to reddish brown and even very dark shades, is reported for particular species growing upon specified substrata The chemical reactions back of these colors are not known for the black aspergilli Blochwitz has reported extractions of color from various species of Aspergillus and other molds and observed that variations in these colors were readily obtained with reagents Some of the extracted material were reported to act as indicators It is therefore, doubtful whether the succession of colors present in such a related series of organisms represent substances differing fundamentally one from another, or merely successive steps in the transformation and reactions of the same general type of product

Occurrence and Economic Importance

The black aspergilli are probably more common than any other representatives of the genus They are world wide in distribution and occur

in and upon the greatest variety of substrata including grains forage products spoiled fruits and vegetables exposed cotton textiles and fabrics leather dairy products and other protein rich substrata and decaying vegetation in the field They are abundant in all soils examined, and from studies which have been made by the authors and other investigators, it would appear that they are particularly abundant in soils from tropical and sub-tropical areas

With the possible exception of the *A flavus-oryzae* group which is of great economic importance in the Orient the black aspergilli are undoubtedly more widely used in industry than any other group of molds Since the present volume is primarily a manual designed to assist the worker in the study diagnosis and maintenance of the aspergilli that come into his hands no attempt will be made to discuss the various fermentations and other biochemical activities of the black aspergilli However these fermentations are of great importance and will be briefly noted For the reader who is interested in these fermentations or perchance is actually conducting them, a fairly complete list of references is presented for each in the Topical Bibliography (Chapter XXII) In each case it has been our aim to present sufficient references to provide the reader with a reasonably comprehensive guide to the literature of the field

Gallic Acid Raulin and his coworkers in Paris during the early 1860 s identified the organism active in the production of gallic acid by the fermentation of gallnuts and other tannin bearing substances The species was first discussed as *Ascophora nigrans* Van Tieghem in 1867, named and described the organism correctly as *A niger* Recurrent investigations have been conducted on this fermentation from that period to the present time (see Topical Bibliography p 294)

Citric Acid In 1917 Currie published his fundamental studies on the formation of citric acid by strains of *A niger* and thereby established the basis for one of the most important of all industrial mold fermentations Subsequent to this investigators in the United States and abroad have made many additional and important contributions While no attempt will be made to cite all of these the works of Bernhauer Wehmer Doelger and Prescott and the U S Department of Agriculture group including Wells May Moyer Herrick and Ward are considered to be outstanding A selected list of references to the citric acid fermentation is presented on pages 290-293

Fumaric Acid Certain strains of the *A niger* group produce appreciable amounts of fumaric acid and it was to one of these forms that Wehmer in 1918 applied the name *A fumaricus* At the present time however fumaric acid is produced in industry by fermentation with species of Rhizopus rather than strains of the black aspergilli (see p 293)

Gluconic Acid Selected strains of *A niger* are used industrially for the production of gluconic acid This process, like the citric fermentation, has been investigated by many workers Outstanding contributions have been made by Molliard Bernhauer, and the U S Department of Agriculture group, including Herrick May, Wells, Moyer, Gastrock, Porges, and others (see pp 295-297)

Oxalic Acid Under certain conditions some strains of *A niger* produce appreciable quantities of oxalic acid While it is usually avoided rather than encouraged, this fermentation has been investigated by Wehmer (1891 and 1892) Raistrick and Clark (1919) Jacquot (1938) and others (see pp 298-299)

The production of these various acids in quantities sufficient to have economic importance represents to an appreciable degree specific strain characteristics and the greatest possible care must be exercised in maintaining these strains in a state of high productivity It has been found however, that in certain cases the same strains can be made to produce substantial yields of two or more of these acids by varying the composition of the nutrient solution and certain environmental factors The reader is referred to the extensive literature on this subject

Enzymes While they are not commonly cultivated for the production of enzymes as such as are members of the *A flavus-oryzae* group, the black aspergilli produce a number of enzymes in appreciable quantities Beginning with Fernbach in Germany (1890) and Bourquelot (1893) in France, various authors have devoted considerable study to their formation A number of papers relative to enzyme production by members of the group are cited in the Topical Bibliography under the subtitle Enzymes of *Aspergillus niger'* (pp 294-295)

Fat Production The mycelium of *A niger* contains appreciable fatty materials (Pontillon 1932, Bernhauer and Patzelt 1935 Schmidt 1935 and others) The waste mycelium from the citric acid fermentation is reported to provide a satisfactory source of sterols for irradiation in the production of Vitamin D

Soil Testing A niger has been successfully employed as an assay organism for determining mineral deficiency of soils particularly deficiencies in phosphorus and potassium Papers on the so-called *A niger* method of soil testing were first published by Kiessling and Schmidt and by Schlots Smith and Brown in the same year (1932) to be followed by more elaborate studies by Stock (1933) Niklas and associates (1933) and others Additional references to this method are presented in the Topical Bibliography (pp 313-314) The strain employed by Niklas is maintained in the culture collection of the Northern Regional Research Laboratory as No 323

Mildew Strains of *A niger* represent a common cause of mildew on exposed wood surfaces and cotton fabrics Partansky and McPherson (1940) used a strain of this species successfully for testing the mold resistant properties of oil paints *Aspergillus niger* is commonly included in the mixtures of miscellaneous molds used for testing the effectiveness of mildew and rot proofing agents when impregnated in textiles and fabrics Where pure cultures are employed species of *Chaetomium* and *Metarrhizium* are generally used

Mold Physiology

Members of the *Aspergillus niger* group have been used extensively in investigations on mold physiology, probably more than any other form As early as 1909 Latham studied nitrogen assimilation by *A niger* (*S nigra*) to be followed in 1911 by Dox studying phosphorus assimilation by the same species Beginning in 1918 and continuing up to the present time, Steinberg has published a succession of papers on the physiology of *A niger* with special reference to the rôle of heavy metals in its nutrition A single strain of *A niger* which is carried in the NRRL collection as No 334 (Thom No 4247) has been used throughout these investigations Studies of a somewhat similar character have been conducted by Bortels (1927), Levy (1932) Gollmick (1936) and others Citation of these papers together with many additional references are presented in the Topical Bibliography under the subtitle Physiology An attempt has been made to present sufficient references to serve as a point of entrance to the literature of the field

Pathogenesis

Members of the *Aspergillus niger* group are commonly isolated from the external ear of man this being the source of the classic *Sterigmatocystis antacustica* of Cramer Other species reported to have been isolated from cases of otomycosis include *A niger* van Tieghem *A phoenicis* (Cda) Thom and Church (with long primary sterigmata) *A giganteus* (Mattlet) Dodge and *A Macfiei* Dodge *A Macfiei* showed no signs of pathogenicity when tested on experimental animals There is no morphology to distinguish either of the latter two forms from the ubiquitous saprophytic types and it appears probable that many strains can become established in the auditory canal under certain favorable and probably temporary conditions

Once entrenched in the flesh about the auditory canal the mycelium has been found to be very persistent One case is known in which occasional abscesses have occurred over a period of 25 years during which desultory treatment has quieted the inflammation but has not destroyed the parasite

In the same way, extensive development of these forms as points of infection in the lungs may give rise to conditions diagnosed as pseudotuberculosis which may persist for long periods without resulting either in death or complete recovery of the patient Spores of *A niger* are air borne and hence are commonly drawn into the respiratory tract They are occasionally reported as causative agents in allergic reactions

Single Strain Cultures

The great importance of some of the black aspergilli as fermentative agents makes punctilious preservation of the actual strain employed the only means either of insuring a process against breakdown or of introducing it in a new locality Even when such a culture is maintained with the utmost care natural variation sometimes occurs Induced variation, such as that described by Steinberg and Thom in a series of papers (1939–40) presents a further hazard in processes where cultures are grown at extreme *H* ion concentrations, or in the presence of chemical or other substances which may actually be toxic at the concentrations employed In a process in one laboratory a strain appeared that presented a colony aspect in which *only a few typical black heads developed in a background of dwarfed and* fractional conidial fruiting structures In another case a mutant appeared which differed so radically from *A niger* in its secondary sterigmata and spore chains that the Yuills described it as a new genus and species *Cladosarum olivaceum* (Trans Brit Myc Soc 22 194–200, Pls 11–13 1938)

The user of one of these Aspergilli then, needs to know his organism in cultural aspect, in microscopic details and in essential reactions in standardized and reproducible substrata Furthermore, he must be able to maintain it free from contamination with other species, and likewise free from such variation, either natural or induced, as will interfere with consistent and controlled results

Chapter XVIII

THE ASPERGILLUS WENTII GROUP

Outstanding Characters

Conidial heads large typically globose often splitting irregularly in age—varying in color from dull yellowish to ecru-olive and from light to dark brown depending upon the species and strain

Conidiophores smooth walled or nearly so but often appearing finely roughened when examined in dry mounts

Vesicles globose fertile over the entire surface Sterigmata in two series

Conidia commonly elliptical smooth or somewhat roughened depending upon the species

Sclerotia present or lacking dark brown to black characteristically white tipped when young

The *Aspergillus wentii* group as presented here is recognized as somewhat artificial in comparison with such strictly natural groupings as the *A glaucus* *A nidulans* and *A clavatus* groups The degree of relationship between the species included is open to question Yet all of the forms possess certain characteristics in common (1) conidiophores are smooth walled or nearly so (2) conidial heads are large and strictly globose at least when young and (3) all appear to occupy taxonomic positions somewhat intermediate between the *Aspergillus niger* group on the one hand and the *Aspergillus flavus* or *Aspergillus ochraceus* groups on the other

Group Key

I Conidia smooth walled
- A Sclerotia lacking vegetative mycelium and young conidiophores reddish in color
  - 1 Conidial heads light brown near wood brown (Ridgway Pl XL) — *A panamensis* Raper and Thom
- B Sclerotia present dark brown to black vegetative mycelium colorless and young conidiophores colorless or pale yellow
  - 1 Conidial heads dull yellow to ochraceous sclerotia globose or nearly so — *A alliaceus* Thom and Church
  - 2 Conidial heads in yellow green shades near ecru-olive (Ridgway Pl XXX) — *A avenaceus* G Smith

II Conidia more or less echinulate
- A Sclerotia present or lacking depending upon the strain and the substratum conidial areas in orange brown to brown colors
  - 1 Conidiophores colorless colonies often conspicuously floccose — *A wentii* Wehmer
  - 2 Conidiophores brown (See *A niger*) — *A hennebergi* Blochwitz

Aspergillus panamensis Raper and Thom in Mycologia 36 568-572 fig 5 1944

Colonies on Czapek s solution agar at room temperature very thin consisting of a sparse and transparent growth of vegetative hyphae almost wholly submerged bearing widely scattered erect conidial structures with radiate heads light brown in color Colonies upon malt extract agar at room temperature growing well and fruiting luxuriantly reddish brown in color, consisting of a dense basal mycelium, predominantly red, from which develop massed condial structures in broken or continuous concentric zones (fig 65 A) many conidiophores abortive and sterile, fertile conidiophores bearing globose to radiate heads light brown in color, near wood brown (Ridgway, Pl XL), these, together with red-colored sterile structures and aerial hyphae give the colony its characteristic appearance and color, in age, colonies tending to develop a loose floccose overgrowth, more or less obscuring the abundant conidial heads reverse dull brown odor none

Conidial structures arising directly from the substratum scattered or abundant, depending upon the culture medium employed (fig 65 B) Heads typically globose in age characterized by loosely radiating chains of conidia, less commonly by few to several roughly columnar masses variable in size, commonly ranging from 250 to 450μ in diameter occasionally up to 500μ varying in color from avellaneous to wood brown (Ridgway, Pl XL) to Saccardo's umber (Ridgway, Pl XXIX) Conidiophores straight mostly 600 to 900μ in length by 9 to 12μ in diameter occasionally larger, with walls smooth, comparatively heavy, ranging from 3 to 3 5μ thick in the basal area to 1 5 to 2μ in the terminal area approximately uniform in diameter throughout except for a limited reduction immediately beneath the vesicle Vesicle colorless comparatively thin walled globose or slightly elongate mostly 25 to 30μ in diameter fertile over the entire area (fig 65 C) Sterigmata in two series closely packed primaries 5 5 to 6 5μ by 2 4 to 2 8μ secondaries 5 to 6μ by 1 5 to 2μ Conidia light yellowish brown in mass globose to subglobose smooth walled mostly 2 2 to 2 6μ in diameter occasionally 2 8μ

Type culture NRRL No 1785 was isolated in January 1912 from Panama soil collected by Mr John T Bonner A second culture NRRL No 1786 differs from the above strain in minor details but clearly belongs with it This was isolated from a second sample of Panama soil collected by Bonner

The species is considered to represent a form somewhat intermediate between the *Aspergillus niger* group and *A wentii* Superficially at least, there is evidence of relationship with *Aspergillus niger* mut *cinnamomeus* (syn *Aspergillus cinnamomeus* Schiemann) and *Aspergillus niger* mut *Schiemanni* (syn *A fuscus* Schiemann) It bears a certain

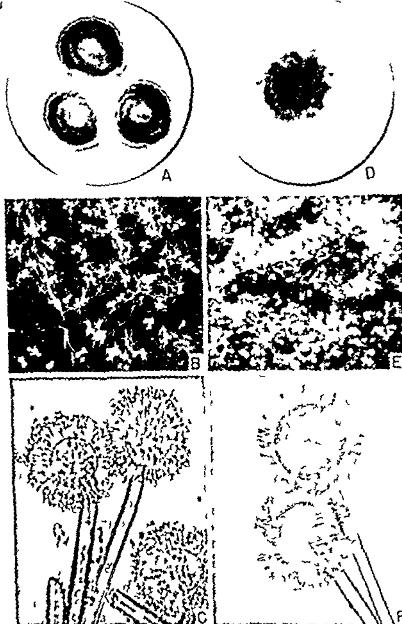


Fig 65 *A–C Aspergillus panamensis* NRRL No 178_ *A* Colonies upon malt extract agar 10 days *B* Conidial heads showing tendency to split into loose divergent columns × 9 *C* Conidial heads showing globose vesicles and sterigmata in two series × 500 *D–F Aspergillus arenaceus* NRRL No _17 *D* Single colony on Czapek's solution agar showing numerous large sclerotia *E* Portion of above colony showing greater detail of heads and sclerotia × 6 *F* conidial heads showing large globose vesicles × 150

resemblance to these forms in the comparatively light color of its conidial heads and in the smallness and general character of its conidia, but differs from these forms in three very striking particulars (1) It characteristically develops an extensive red colored aerial mycelium upon media such as malt-extract agar where it attains its maximum growth, (2) it grows very sparsely upon Czapek's solution agar upon which the above noted forms grow luxuriantly, and (3) it possesses very small primary sterigmata, measuring 5 5 to 6 5μ by 2 4 to 2 8μ in contrast to 13 to 15μ by 3 to 5μ for mut *cinnamomeus* and 15 to 40μ by 4 6μ for mut *Schiemanni* Whether or not the species actually represents a naturally occurring mutation from *A niger* can only be guessed The smallness of its conidia and primary sterigmata would hardly support this hypothesis In cases where mutations have been obtained from known cultures the dimension of specific structures in such mutations generally agree very closely with those of the same structures in the parent strain, and black Aspergilli with the dimensions of *A panamensis* are rarely, if ever, encountered in nature The possibility of this representing a mutation is not excluded, but until additional evidence supporting such origin is forthcoming the writers feel warranted in maintaining as a distinct species this unique form which obviously is able to maintain itself in the soils of Panama

The correct taxonomic position of this species remains in doubt It is included with *Aspergillus wentii* although we realize that this placement is not entirely satisfactory As continued isolations are made from tropical soils and other sources it is our hope that additional forms may be found, which will furnish evidence of a more exact relationship

The very sparse development of *A panamensis* upon Czapek's solution agar containing sucrose results from an invertase deficiency, when dextrose is substituted as a carbon source the fungus grows luxuriantly and fruits abundantly

Aspergillus alliaceus Thom and Church, in The Aspergilli p 163 1926
Discussed without name as Thom No 4660 by Walker and Lindegren, in Jour Agr Res 29 507–514 1924, and by Walker Lindegren, and Bachmann, in Jour Agr Res 30 175–187 1925

Colonies on Czapek s solution agar rapidly and broadly spreading with loosely floccose aerial sterile mycelium bearing scattered ochraceous heads among abundant dark to almost black sclerotia (Pl VI D and fig 66 A) in some strains, predominantly floccose with limited conidial heads and few sclerotia in others (fig 66 B) reverse uncolored Conidial heads dull yellow to ochraceous strictly globose when young and remaining radiate or splitting irregularly in age (fig 66 D) up to 300μ in diameter often more abundant in cultures after many transfers Conidiophores up to 150μ

long by 15μ with walls up to 1 5μ in thickness appearing smooth in liquid mounts but showing rudimentary markings or pits when examined dry Vesicles globose to subglobose up to 40 to 50μ in diameter, with walls about 2μ in thickness and showing pores where sterigmata are attached Sterigmata in two series, primary 7 to 9μ or even 12μ by 3 to 4μ secondary

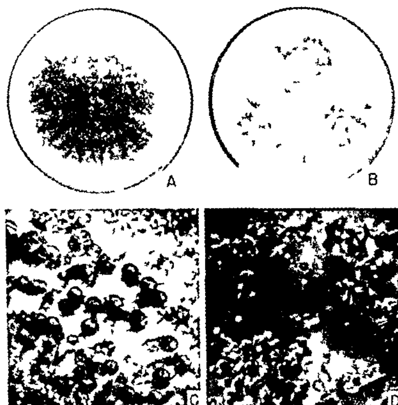


FIG 66 *Aspergillus alliaceus* A Heavy sclerotium producing strain NRRL No 315, on Czapek s solution agar 10 days B Light sclerotium producing strain NRRL No 318 growing under similar condition C Portion of A showing greater detail of sclerotia and conidial heads × 9 D Conidial heads from old culture (strain NRRL No 318) showing tendency to split into divergent columns × 18

7 to 8μ by 2μ Conidia elliptical to globose, yellowish 2 5 or 3μ in diameter Perithecia not found Sclerotia often very abundant (fig 66 C) at first white but quickly becoming black or nearly so in many strains dominating the character of the colony

Strains compared include NRRL No 315 (Thom No 4656) from a dead

blister beetle (*Macrobasis albida*), NRRL No 316 (Thom No 4660) isolated from onion bulbs and others with characteristic heads and sclerotia have been obtained from garlic bulbs from cactus plants and from soils particularly from the general region of Texas, Arizona and Mexico Strains have also been isolated from soils collected near Calcutta

The large globose, pale yellow to ochraceous heads of the species strongly suggest relationship to the *A ochraceus* group The smooth walled conidiophores and black sclerotia however, more closely ally it with *Aspergillus wentii* and the other species grouped with it It may however represent a form somewhat transitional between the great groups represented by *A niger* on the one hand and *A ochraceus* on the other

Aspergillus avenaceus Geo Smith in Brit Mycol Soc Trans 25 24-27, *Pl 1, figs 1-3 1943*

Colonies on Czapek s solution agar (with sucrose) at room temperature spreading rapidly, more or less conspicuously zonate (fig 65 D), slightly floccose white at first then dull yellow to ecru-olive (Ridgway, Pl XXX) as shown in Pl VI E, with, at times, a greenish tinge without becoming truly green, reverse pale dirty pink Conidial heads large globose 400 to 600μ in diameter, or up to 1 000μ, splitting into columnar masses of conidial chains Conidiophores up to 5 mm long 18 to 30μ in diameter with walls 2 5 to 4μ thick, smooth in fluid mounts, but appearing finely roughened when examined dry Vesicles globose or slightly flattened thick walled up to 185μ in long axis sterigmata in two series primary 22 to 50μ by 6μ secondary 11 to 13μ by 4μ (fig 65 F) Conidia ellipsoid smooth 4 to 6 or 6 5μ by 3 2 to 4μ Sclerotia dark grayish brown to black (fig 65 E) elongate irregularly flask shaped sometimes with the neck" forked, apical portion white to gray during development 2 to 3 mm in long axis scattered in concentric zones after 7 to 10 days

On Czapek agar with glucose, sclerotia are more abundant and larger On wort or potato agar, conidial heads are abundantly produced but sclerotia are delayed for several weeks and are few in number

Species characterization adapted from George Smith s description

This very distinctive species (NRRL 517 Thom 5725) is represented by a single isolation from seed peas made in 1938 by Dr G E Turfitt of the London School of Hygiene and Tropical Medicine University of London

Aspergillus wentii Wehmer in Centralbl f Bakt etc 2 Abt 2 p 150 1896 See also The Aspergilli Thom and Church p 183 1926

Colonies on Czapek s solution agar rapidly growing and broadly spreading floccose with white or yellowish aerial hyphae which in some strains pile up in the plate (Pl VI F and fig 67 A) or fill the test tubes for several

centimeters (fig 67 C) but remain inconspicuous in other strains (fig 67 B), with developing heads at first white through yellow shades to olive brown medal bronze or snuff brown (Ridgway Pls IV XVI, XXX column 19, and XXIX column 15 K) or according to Wehmer, coffee brown to chocolate brown reverse becoming reddish brown in old cultures Conidial heads large globose generally remaining radiate in age (fig 67 D) ranging up to 500µ in diameter, changing from yellow shades to brown Conidiophores up to several millimeters in height by 10 to 25µ in diameter with walls colorless up to 4µ in thickness studded with droplets in growing colonies and often appearing slightly roughened when examined dry, but uniformly smooth in fluid mounts Vesicles globose or nearly so (fig 67 E) varying up to 80µ in diameter fertile over the entire surface Sterigmata usually in two series primaries 10 to 20µ by 3 to 5µ occasionally much larger secondaries 6 to 8µ by 3µ Conidia borne in long chains more or less elliptical ranging from 3 5 to 6µ in long axis but mostly 4 to 5µ double wall clearly evident ranging from almost smooth to marked by ridges sometimes suggestive of *A niger* again more closely resembling the *A flavus* series No perithecia reported Sclerotia often encountered (fig 67 F) dark brown to black ovate with long axis vertical

Culture description based upon strain NRRL No 375 (Thom No 116) obtained in 1909 from the Centraalbureau as Wehmer s original organism as well as numerous isolations from soils and other materials collected by the authors in the United States and other strains contributed by investigators from all over the world

Numerous strains with the general aspect of Wehmer s species have been seen from Java China South America Japan the Straits Settlements British Guiana and Brazil In our experience it has been isolated from cottonseed cake from olives from soil and from numerous other sources It is to be regarded as very widely distributed and to be common on many types of decaying vegetable products

The variations in colony aspect in different strains run from an extreme of mycelial growth filling the test tube that is characteristic of cultures such as the Wehmer organism to colonies forming a crowded surface growth of conidiophores only and distinguishable from *A tamarii* only by a lack of greenish color in the early fruiting period and in the characteristic smooth conidiophores and finely roughened conidia

Aspergillus archaeoflavus Blochwitz (Ann Mycol 31(1/2) 73 83 1933) represents *a non floccose form which is hardly separable from A wentii In our examination* of the type strain (NRRL No 382 Thom No 5346) received in 1933 from Baarn measurements were somewhat less than those cited by the author The absence of conidial markings to which Blochwitz called attention would not bar it from *A wentii* since in some strains conidia are almost entirely smooth in others finely roughened while in still others they are conspicuously echinulate

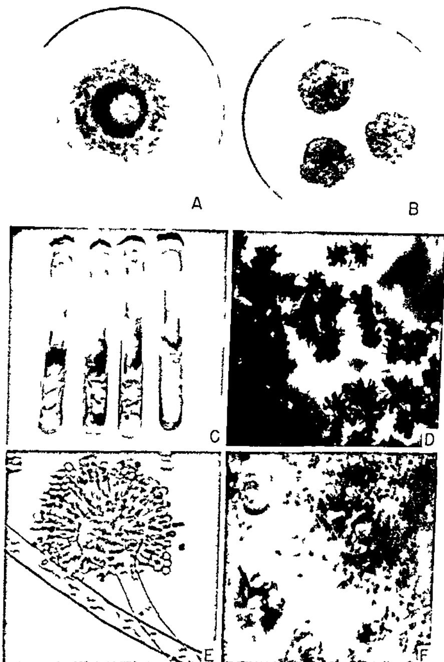
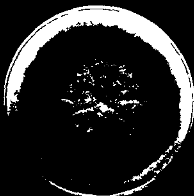


Fig 67 *Aspergillus wentii* A and B colonies of strains NRRL No 375 and No 385 respectively upon Czapek's solution agar 10 days C Tube cultures of four representative strains D Conidial heads from old culture × 18 E Single head showing smooth conidiophore globose vesicle and sterigmata in two series × 500 F Detail of colony margin in strain NRRL No 379 showing sclerotia and abundant conidial heads × 9

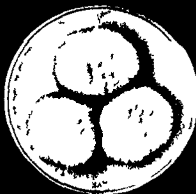












P VII

A (ppe l ft) *A pe gill terricola mericana* M rchal NRRL N 424 *B* (ppe gl t) *A pe g'll tamarı* K ta NRRL N 4 *C* (ce t lef) *A pergll oryza* (Ahb) Coh NRRL N 459 *D* (ce t ht) *A pe ll fl* L k NRRL N 1957 *E* (l l ft) *A pe g llus que ci us* (B) Th d Ch) NRRL N 394 *F* (l ht) *A pe g ll och ac* W ll lm, NRRL N 319 All lt res ro po C pek ag l t g (C) ph to raph b H es N rth rn R g nal Rese rch Laboratory Reprod ced thro gh co-operat of Chas Pfi & Co I)

Aspergillus wentii var *minimus* Nakazawa Takeda Okada and Simo (Jour Agr Chem Soc Japan 10(2) 176–177 1934) shows measurements varying somewhat from those of the species and from those given by Blochwitz but it is not sufficiently marked to warrant separation

Sterigmatocystis aerea Bainier (Bull Soc Bot France 27 28 1881) may have been a member of this series but was not sufficiently described to permit positive identification

Aspergillus hennebergi Blochwitz in Ann Mycol 33 235–239 1935 The species is described as having the aspect and colors of a non floccose *A wentii* or an *A tamarii* with red sclerotia but with conidiophores browned as in the partially browned conidiophores of the *A niger* group

An albino variant of *A wentii* was isolated by Mosseray (Ann Soc Sci Brux 54 161 189 1934) from a normal culture of this species and was found to retain its distinctive characters through repeated transfers in laboratory culture No name was given to this mutant

Occurrence and Economic Importance

Aspergillus wentii is a cosmopolitan species that is fairly common in soils upon moist grains and other vegetable matter undergoing slow decomposition and may be isolated less frequently from a wide variety of other materials collected from nature It is apparently world wide in distribution In the Orient it is often included with *Aspergillus tamarii* *A flavus* and *A oryzae* all under the latter name as a rule in the Koji' preparations used in the manufacture of various soy products Likewise it has been investigated with these same species in connection with the production of various mold enzymes At the same time it has been included with the black Aspergilli in studies on the production of organic acids by molds Recently Karow (1942) has reported one strain of this species to give substantial yields of citric acid in submerged culture Yabuta (1912) reports Koji acid production by *A wentii* On the whole strains of the species appear to be somewhat less active biochemically than either the black Aspergilli or members of the *A flavus-oryzae* group It is nevertheless a vigorously growing species with definite biochemical possibilities and should not be overlooked in any program relating to mold fermentation

Aspergillus alliaceus appears periodically upon alliaceous bulbs and occasionally upon cacti as at least a secondary parasite It is not infrequently isolated from soils and appears to be fairly common in the southwestern states of Texas New Mexico and Arizona It has also been isolated from soils of other areas including Southern Mexico and India Nothing is known regarding its biochemical possibilities

Only the type strains of *A avenaceus* and *A panamensis* are known and neither has been shown to have any economic importance

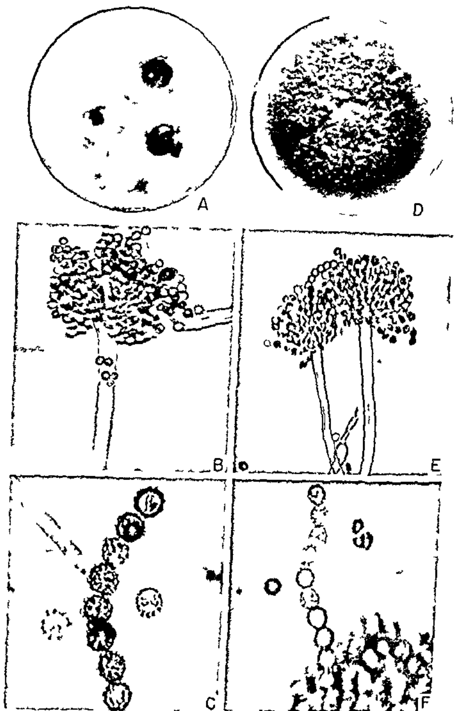


FIG 68 *A–C Aspergillus lutescens* NRRL No 426 *A* Colonies on Czapek's solution agar showing extreme floccose habit and light sporulation 10 days *B* Single conidial head × 500 *C* Conidia × 1200 *D–F Aspergillus terricola* NRRL 424 *D Single colony on Czapek's solution agar showing somewhat floccose* but heavily sporing colony 10 days *E* Conidial heads showing sterigmata in a single series × 500 *F* Conidia × 1200

in one series in smaller and crowded heads up to 15 to 20μ by 4 0 to 5 0μ sterigmata often in two series in larger heads with primaries about 15 to 18μ by 4 to 5μ secondaries 12 to 14μ by 4 to 5μ Conidia subglobose varying from 5 by 7μ to 8 by 9μ conspicuously roughened with prominent tubercles of color (fig 68 C)

The species is known only in the type culture from the Bainier collection NRRL No 425 (Thom No 4640 478) and as a second strain NRRL No 426 isolated in the Soil Microbiology Laboratory Bureau of Plant Industry Washington D C about 1939

Aspergillus terricola Marchal in Rev Mycologique 15 No 59 101–103 1893

Colonies umbrinus mycelial hyphae 3 to 5μ in diameter without anastomoses conidiophores hyaline continuous or septate in age 600 to 1 000μ by 7 to 10μ (whole depth of colony growth) vesicles subglobose hyaline 39 to 50μ radiately covered with sterigmata sterigmata in one series 12 to 15μ by 4 to 7μ conidia umber (Sacc) ovate or elliptical then globose rough with colorless connectives

Description from Marchal of a culture isolated from soil in Belgium not reported elsewhere see variety below

Aspergillus terricola var *americana* Marchal cultural description by Thom and Church in Am Jour Bot 8 125 1921

Colonies on Czapek s solution agar growing rapidly at room temperature often somewhat floccose in central colony areas (fig 68 D) ranging from shades near yellow ochre (Ridgway Pl XV) when young to Dresden brown or mummy brown in age near Saccardo s umbrinus (Pl VII A) aerial growth largely consisting of crowded conidiophores reverse uncolored Conidial heads radiate hemispherical to subglobose loose in texture consisting of comparatively few divergent chains of conidia up to 200μ in diameter Conidiophores 300 to 600μ in length by 6 to 8μ in diameter with walls pitted Vesicles globose to subglobose (fig 68 E) up to 25μ in diameter fertile over the upper two thirds or three fourths Sterigmata in one series 7 to 10μ by 2 to 4μ Conidia tuberculate (fig 68 F) from the presence of color bars variously distributed between the outer and inner wall ovate to nearly globose from 3 by 5μ up to 5 by 7μ usually about 5 5μ occasionally 5 to 8μ in diameter

Type culture NRRL No 424 (Thom No 4838) isolated by F M Scales from redland soil in Georgia and discussed by Scales in Jour Biol Chem 19 459–472 1914 under the name *A terricola* Marchal Scales culture was submitted to Marchal who designated the form as *A terricola* var *americana* Marchal distinguished as follows The dimensions of the

vesicles 14 to 20μ instead of 30 to 50μ of the sterigmata 5 6 to 10 5μ by 2 2μ instead of 12 to 15μ by 4 to 7μ the spores only very delicately verrucose, separate your fungus from *A terricola*'

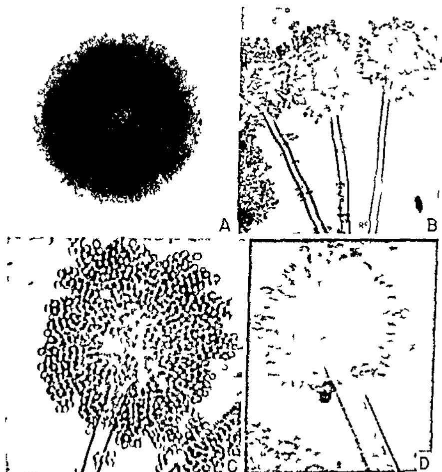


FIG 69 *Aspergillus tamarii* *A* Colony growing on Czapek s solution agar characterized by heavy conidial production strain NRRI No 427 10 days *B* Conidial heads × 160 *C* Single head × 300 *D* Single head further enlarged showing rough walls of conidiophore and sterigmata in two series × 500

Aspergillus tamarii Kita in Centralb f Bakt etc 2 Abt **37**, No 17/21, pp 433–452 1913 Characterization of *A tamarii* Kita given by Thom and Church in Am Jour Bot **8** 118 1921 See also Thom and Church The Aspergilli p 194 1926

Colonies on Czapek s solution agar spreading broadly at room temperature (fig 69 A) with vegetative hyphae mostly submerged fruiting areas

at first colorless then passing through orange yellow shades to brown in old colonies variously Isabella color light brownish-olive buffy-citrine medal bronze or raw umber (Pl VIII B) (Ridgway Pls XXX XVI and IV Column 19 and Pl III Column 17) not showing true green but often presenting a suggestion of green that is transient and limited to areas of young heads reverse uncolored or occasionally pinkish Conidial heads varying greatly in size in the same fruiting area from more or less columnar to nearly but not completely globose and up to 300μ in diameter with radiating chains and columns of conidia Conidiophores arising from submerged hyphae up to 1 to 2 mm in length colorless with walls becoming abruptly thinner at the base of the vesicle frequently showing irregular thickenings within as a rule markedly rough or pitted throughout part or all of their length (fig 69 D) sometimes appearing smooth or nearly so when examined in liquid mounts but consistently rough or pitted when examined dry Vesicles globose to subglobose 25 to 50μ in diameter (fig 69 C) with fairly thin walls which frequently crush in mounts fertile over almost the entire surface Sterigmata in one series in small heads in two series in large heads primary sterigmata commonly 7 to 10μ by 3 to 4μ becoming 20 to 35μ long in gigantic heads secondary sterigmata 7 to 10μ by 3μ Conidia ranging from more or less pyriform through subglobose to globose conspicuously roughened from prominent tubercles and bars of orange yellow coloring matter deposited between the loose outer wall and the firm inner wall commonly ranging from 5 0 to 6 5μ in diameter occasionally up to 8μ Sclerotia produced by many strains usually purplish or reddish purple globose to pyriform with apex white

Species characterization is based upon Thom and Church's culture No 4235 12 (NRRL No 429) which was submitted to and identified by Kita as *A tamarii* (see Thom and Church Am Jour Bot 8 118 1921)

The organism described by Kita proved to be one of a great series represented in our collection It is common among cultures examined from North and South America from Japan China India and from Europe The species has been found to be quite common in soil collected from many areas in the United States

The outstanding characters are orange yellow to brown colonies coarse colorless conidiophores usually roughened but with this character sometimes obscure large radiate loose textured conidial heads sterigmata in one or two series commonly with single and double sterigmata in the same head conidia more or less pyriform 5 to 8μ in long axis with tubercles or bars of orange yellow coloring matter between the inner and the outer cell walls

In preparing the manuscript for The Aspergilli (1926) Thom and Church introduced into their general key without name on page 248 under No 279 a series of forms with morphology and general appearance

bridging the gap between *A tamarii* Kita and the *A flavus* group Without publication they referred to these as 'The Bronze Series These strains have the yellow green color of *A flavus* during the early stages of their development, but subsequently develop the yellow to brown colors of *A tamarii* Recognition of this border group is necessary since some strains of *A tamarii* do not assume a definitely green color at any state in their development, while others show green as a transient character Strains of *A flavus*, on the other hand, are typically characterized by the green to yellow green colors Furthermore, the conidia of *A tamarii* are typically quite roughened, showing prominent tubercles or bars of coloring matter deposited between the outer and inner walls In contrast, the conidia of *A flavus* are less coarsely roughened and show more numerous and smaller tubercles or echinulations as well as a greater tendency for the coloring substance to be generally diffused throughout the spore envelope The forms under consideration show in some degree the coloration and spore characters of both groups and are believed to be truly intermediate between *A tamarii* and *A flavus*

The fact that we received from Baarn in 1933 as Blochwitz's *A luteo virescens* Bloch (Ann Mycol **31** 73–83 1933) a culture (Thom No 5345) which represented satisfactorily this intermediate series is not accepted as justifying the assignment of this name to the series since the morphological characters displayed by the strain were so completely at variance with the original description, and since Blochwitz considered his species to be close to *A ustus* We question whether any sharp line of separation can be drawn between the two series because of the repeated appearance of intermediate forms While we do not feel justified in assigning to these forms any specific designation we do feel obligated to continue to call attention to their existence We have at times considered the desirability of moving the whole *A tamarii* complex over into the *A flavus-oryzae* group, but this course has been abandoned since it was felt that to do so would introduce into an otherwise perfectly integrated group a series of organisms whose relationship to them while strongly suggested is not proved and which in its typical form would introduce discordant features

The species listed below are believed to represent probable synonyms

Biourge attached the manuscript name *A vulpinus* to a member of the *A tamarii* series (Thom No 4733 146) and contributed it to our collection but it does not seem sufficiently different from the species to warrant separation

One strain of *A tamarii* was found in the Bainier collection (Thom No 4640 397) as *A cacao* nomen nudum another under the same name came from Pribram

A gigas Spegazzini Myc Argent V in An Mus Nac Buenos Aires Ser II Tome 13 424 1911 was described from decaying coffee leaves in terms that suggest its relationship to *A tamarii*

A spadix Amons in Archief voor de Suikerindustrie in Nederlandsch Indie Jaarg 29 Deel 1 pp 12 14 Jan -June 1921 From the description this is a synonym of *A tamarii* Kita Colonies described as yellow brown to deep brown growing well one ommon laboratory media without aerial mycelium in reverse colorless rice colored to light violet at first then light fuscous brown conidiophores up to 2 to 3 mm by 8 to 9μ with walls about 0 9μ thick and pitted or rough vesicles globose up to 50μ in diameter or almost clavate in small heads conidia 5 5 to 7 2μ rough Culture Amons Not studied by us

A erythrocephalus B and C in Jour Linn Soc (London) Bot 10 362 1869 (See Fungi Cubensis Wrightiana 1868 Type No 642 in Curtis Herbarium deposited in the Cryptogamic Herbarium of Harvard University bears Wright s No 764 Part of the original material was removed by Dr Farlow and given to Thom for study)

Microscopic examination of this type specimen gives measurements as follows conidiophores 45 to 70μ in diameter up to 2 mm in length with walls very heavy 5 to 12μ thick varying from 5 to 6μ in the broader part to 10 to 12μ at the narrower base pitted or roughened vesicles up to 100μ in diameter nearly globose fertile all over head washed free from spores about 150μ in diameter sterigmata in two series primary 8 to 10μ in length secondary 8 to 9μ in length conidia commonly 8 by 6μ ranging up to 8 to 12μ by 5 to 9μ finely pitted or roughened with rather thin walls Colors in the material are questionable on account of the age of the collection

Cultures None Type material only known Placed between the *A tamarii* and *A flavus* groups The amended description is offered due to the existence of a type specimen with very conspicuous characters under a name only very briefly described in 1869 When grown upon natural substrata such as grains et cetera conidiophores and heads of *A tamarii* become very much larger than those ordinarily produced in culture media This might account for this specimen which bears the name *A erythrocephalus* B and C

Occurrence and Economic Importance

Of the species included in this group of brown spored Aspergilli only *A tamarii* is in any sense widely distributed or common in nature *Aspergillus lutescens* is known only as the type culture and as a second isolation made in Washington D C many years later *Aspergillus terricola* has not been positively identified since its description although the form with smaller heads and less coarsely roughened spores designated *A terricola* ar *americana* by Marchal is occasionally encountered *Aspergillus amarii* is however a cosmopolitan mold upon vegetable material under oing slow decomposition and can be isolated from almost all soils examined ike *A niger* and *A flavus* it is more frequently recovered from warm and semi tropical soils than from cool temperate soils although it occurs in the latter The species commonly appears with *A flavus* and *A oryzae* as a constituent part of the koji used in the fermentation industries of the Orient Certain strains apparently produce appreciable amounts of diastatic and proteolytic enzyme while other strains are known to produce

kojic acid Gould reported this in 1938 and it was subsequently confirmed by A J Moyer (unpublished notes) for a strain isolated from Panama soil at the Northern Regional Research Laboratory in 1941

Kita's culture was isolated from a soybean sauce termed 'Tamari", hence the species name Tamari is made by a shorter fermentation process than soy sauce or shoyu, and differs from it in flavor Kita believed that where it was made empirically it owed its individuality to the particular aspergillus which he isolated and described

CHAPTER XX

THE ASPERGILLUS FLAVUS-ORYZAE GROUP

Outstanding Characters

Colonies varying from very light greenish yellow to deep yellow green (Ivy Green)
Conidiophores rough or pitted colorless
Heads hemispherical to columnar to subglobose
Sterigmata in one or two series often varying in the same head
Vesicles variable in form from hemispherical to dome shaped in small heads to globose in large heads
Conidia more or less roughened
Sclerotia characteristic of many strains generally grayish brown to black entirely lacking in others

Two species names are widely used for members of this cosmopolitan group *Aspergillus oryzae* is applied quite generally without regard to morphology to the strains used by the Japanese and Chinese in the fermentation of rice and soy products Although purified cultures are used in many places the nomenclature is based more upon utilization than upon morphology There appears however in these industries a series of strains with long conidiophores radiate heads mostly greenish yellow with the green often fading completely in old cultures These strains appear to be most commonly used in the production of the diastatic type of ferments and to be distributed in the great culture collections as *Aspergillus oryzae* (Ahlb) Cohn Such strains seem to be mostly oriental or tropical in origin Strains with shorter conidiophores and yellowish green heads on the other hand appear wherever fermenting or decaying materials are examined microscopically or by culture *Aspergillus flavus* Link has been accepted as a species aggregate for this second array of forms from which the segregation of sections for description as separate species has been found difficult if not almost impossible If one wishes to perpetuate species names as roughly covering aggregates of closely related but varying strains bearing always in mind that no sharp lines of differentiation exist certain applications of names may be made arbitrarily about as follows

Group Key

I Sterigmata mostly in one series double sterigmata also present
A Conidiophores long 1 several mm heads radiate greenish yellow conidia pyriform more or less roughened variable in size up to 6 8 or even 10μ in long axis
A oryzae (Ahlburg) Cohn

B Conidiophores 600 to 1700μ heads radiate hemispherical pale greenish yellow conidia smooth globose 3 0 to 4 6μ *A micro viridi-citrinus* Costantin and Lucet

C Conidiophores mostly less than 500μ heads deep yellowish green (Ivy Green) described as a parasite of the mealy bug of cane occasionally elsewhere *A parasiticus* Speare

II Sterigmata mostly in two series but single series common and often in same head small heads usually showing single series only

A Conidiophores very variable in length mostly 400 to 1000μ heads in various yellowish green shades *A flavus* Link

B Conidiophores mostly borne as short branches from trailing hyphae forming an uneven cottony mass heads white to yellow with traces of green only *A effusus* Tiraboschi

Literally hundreds of strains of this group have been collected and compared Many of them have been isolated from fermentation investigations in the laboratory and from industrial processes No correlation of colony appearance, conidiophore or head morphology, color or microscopic detail, with actual utilization has been proved A culture labeled *A oryzae* (Ahlburg) Cohn, NRRL No 447 (Thom No 113) has been preserved for over 30 years without apparent change in morphology It is probably derived from Cohn's organism When however we scrutinize the Aspergilli obtained from the rice or soy fermentations of the Orient cultures of the type represented by this strain are not the most common The pre eminently useful strains usually have the aspect of forms intermediate between *A flavus* and *A oryzae* The dwarf green *A parasiticus* of Speare isolated from dead mealy bugs of sugar cane in Hawaii proved no more parasitic to the same species of insects in the Barbados than other *A flavus* strains sent with it Teizo Takahashi contributed his series of strains under the letters used in his publication (1913) These are discussed at some length in Thom and Church s paper on *A flavus A oryzae* and associated species (1921) and also in The Aspergilli ' (1926 p 202) It is sufficient to say that they vary all the way from almost white with few lightly colored heads to rich yellow green in which heads are very numerous and fairly dark They vary likewise in the length and diameter of their conidiophores Characters of color and conidiophore length are not always correlated, although it is generally true that the darker conidial masses are borne upon shorter stalks The collections contributed by Oshima Kita Hanzawa and others from Japan as well as those isolated from commercial koji (sold as inoculum for fermentation industries) showed mainly the *A flavus* morphology Strains of this series appear constantly where cultures are made from soil or from decaying vegetation *A flavus* and its allies appear in collections from every correspondent who contributes Aspergilli It is debatable whether the worker will be benefited or confused by the introduc

tion of some of the species names applied to members of the group It must not be forgotten that any variant from the dwarf and deep green *A parasiticus* to the longest stalked and palest greenish yellow *A oryzae* may be found if we look for it

Aspergillus oryzae (Ahlburg) Cohn in Jahresb Schles Gesell Vaterl Cultur (1183) 61 226 Breslau 1884

Synonym *Eurotium oryzae* Ahlb The name *E oryzae* with an incomplete description for the same organism was published by Korschelt in Dingler s Polytechnisches Jour 230 330 1878 as taken from a letter from Herr Ahlburg See also Thom and Church Amer Jour Bot 8 106 1921 and The Aspergilli p 198 1926

Colonies on Czapek s solution agar rapidly spreading with vegetative hyphae mostly submerged and forming a white to gray mycelial layer in the form of a tough felty mass (fig 70 A) developing pale greenish yellow shades with the production of ripening conidial areas varying from lime green to mignonette green (Ridgway Pl XXXI column 25) with the green disappearing later and the general color shifting to yellowish brown shades mycelium and agar uncolored Conidial heads predominantly large abundant globose radiate with chains of conidia separate rather than adhering (fig 70 D) giving the pale yellow shades of the colonies Conidiophores 2 to several mm long by up to 20 to 25μ in diameter with walls rather thin definitely pitted or rough (fig 70 F) colorless Vesicles globose to subglobose less often hemispherical up to 50 or even 70μ with walls 1 to 1 5μ Sterigmata commonly in one series up to 15 or 20μ long by 3 to 5μ or in two series with primary sterigmata up to 12 by 5μ and secondary sterigmata 10 to 12μ by 3 5μ (fig 70 B) Conidia more or less pyriform (fig 70 F) varying greatly in size in the same culture and in different strains 3 by 4μ 4 by 5μ 5 by 6μ or up to 9μ or 10μ in long axis occasionally rather thin walled roughened becoming coarsely and deeply roughened in some strains Sclerotia dark few and not forming clumps produced sporadically under undefined conditions

Diagnosis based primarily on culture NRRL No 447 (Thom No 113) received from the Centraalbureau at Baarn and believed to be derived from Cohn s original strain

While the above description is believed to conform closely to the original conception of *A oryzae* strains possessing the essential morphology described but which are heavier sporing and somewhat darker in color are more commonly encountered Culture NRRL No 458 obtained from Dr Oshima as strain AoOld and shown in Pl VII C and Fig 70 C–F is representative of these forms

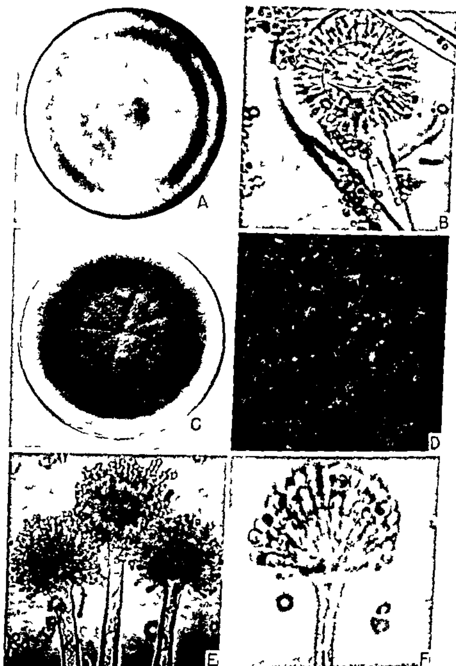


Fig 70 *Aspergillus oryzae* *A* and *B* Strain NRRL No 447 (Thom No 113) *A* Single colony on Czapek s solution agar loose textured conidiophores long and limited in number 10 days *B* Details of single head vesicle thin walled sterigmata mostly in two series × 480 *C–F* *Aspergillus oryzae* NRRL No 458 *C*, Single colony on Czapek s solution agar comparatively heavy sporing 10 days *D* Conidial heads of the same × 18 *E* Conidial heads further enlarged × 270, *F* Single head showing vesicle sterigmata in a single series and roughened conidiophore × 775

The production of perithecia by members of this series was reported by Bezssonoff (1919) without adequate description and by Zikes (1922) whose culture as received from him belonged in the *A glaucus* group No ascosporic form is verifiable for the group thus far

Aspergillus micro-virido-citrinus Costantin and Lucet in Ann Sci Nat Bot (IX) 2 158 1905

The appearance of colonies and measurements of conidiophores heads and spores indicate a form intermediate between *A flavus* and *A oryzae* except for its small conidia The description is very nearly satisfied by Takahashi s culture P (NRRL No 480) It was found to grow between 15 and 45 C and to be pathogenic to rabbits Colonies were greenish yellow to predominantly yellow but contained some definitely green admixture in contrast to *A oryzae* which often lacks green color entirely Conidiophores 600 to 1700μ in length up to 21μ in diameter near the vesicle uncolored granular (= pitted) above smooth toward the base Vesicles 24 to 62μ in diameter Sterigmata varying in size and arrangement with the size of the heads examined Conidia globose smooth 3 to 4 6μ (3 1μ as a minimum to occasional diameters of 5 5μ)

An occasional culture shows the morphological characters described by Costantin and Lucet No actual identity has been proved

Aspergillus flavus Link in Obs p 16 1809 also in Sp Plant 6 66 1824 cited as synonym of *Monilia flava* Persoon Myc 1 p 30

Synonym *Eurotium Aspergillus flavus* DeBary and Woronin in Beitrage zur Morphologie und Physiologie der Pilze III Reihe p 380 1870 Exsiccati by Brefeld preserved in Rabenhorst Fungi Europaei Edit Nov ser II No 2135 one packet in the collection of the New York Botanical Garden

Colonies on Czapek s solution agar spreading rapidly with floccosity limited to scanty growth of sterile hyphae in older and dryer areas among crowded conidiophores conidial areas range in color in various strains from sea foam yellow through chartreuse yellow citron green lime green to Kronberg s green (Pl VII D and fig 72 A) or even to ivy green (See Ridgway Pl XXXI column 25) yellow green colors are either persistent or in old colonies altered by the disappearance of the green factor leaving shades of yellow brown reverse yellowish at first passing over into brown shades in age Conidial heads vary from small with a few chains of conidia to large radiate (fig 72 E) or columnar masses in the same culture and varying mixtures of different types and sizes of head Conidiophores mostly arising from submerged hyphae commonly 400 to 1000μ long by 5 to 15μ in diame

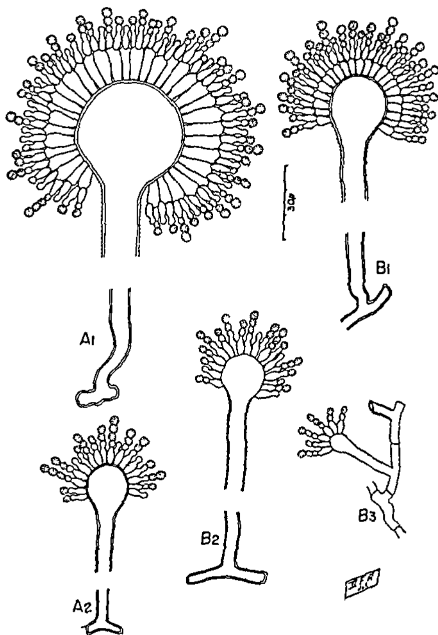


Fig 71 Conidial structures in the *Aspergillus flavus-oryzae* group A *A flavus* NRRL No 482 A_1 typical large radiate to globose head showing sterigmata in two series A_2 small, loosely columnar head showing single series of sterigmata *B A effusus* NRRL No 506 B_1 large radiate head showing double series of sterigmata B_2 smaller head showing sterigmata in a single series *B* diminutive head borne upon one of a chain of foot cells In this group single and double sterigmata often occur in the same head (not illustrated)

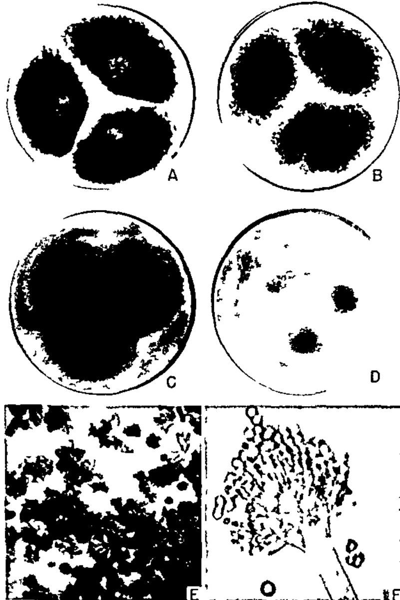


Fig 72 *Aspergillus flavus* *A* Typical strain NRRL No 1957 showing crowded conidial heads and occasional black sclerotia *B* Heavy sclerotium producing strain NRRL No 500 *C*, Strain used for the production of kojic acid NRRL No 484 (This approaches the character of *A oryzae*) *D* Dr White s strain of *A flavus* (NRRL No 501) showing characteristic thin growth and sparse sporulation *E* Typical conidial heads × 18 *F* Single head showing vesicle sterigmata conidia and the roughened conidiophore × 780 All cultures on Czapek s solution agar 10 days

ter with walls pitted, rough (fig 72 F) almost spiny in appearance broadening upward and gradually enlarging into vesicles 10 to 30 or 40μ in diameter, dome like in the smaller heads flask-shaped in larger heads (fig 72 F) Sterigmata in a single series in many smaller heads (fig 71 A_2) or both single and double series on the same vesicles in large heads (fig 71 A_1), varying from single sterigmata only 10 to 15μ by 3 to 5μ, to primary sterigmata 7 to 10μ by 3 to 4μ and a secondary series 7 to 10μ by 2 5 to 3μ Conidia pyriform to almost globose, nearly colorless to definitely yellowish green, varying from 3μ, 3 by 4μ, 4 by 5μ, or even larger and marked variously with pits, echinulations, or irregularly winding color bars and ridges to give a roughened effect of varying intensity Sclerotia, when found, at first white then brown hard parenchymatous and a few strains white tipped produced by *some strains regularly and abundantly (fig 72 B)*, scantily by others under undefined conditions Perithecia not found

Description originally based upon culture NRRL No 482 (Thom No 108) from the Centraalbureau at Baarn Holland but supplemented by observation of many hundreds of cultures from many substrata and all parts of the world

Unless segregation under a specific name is supported by adequate morphological and reproducible cultural data there is no way to identify the organisms intended Applying these criteria no reasons are seen for the use of the following specific designations

A wehmeri Constantin and Lucet in Ann Sci Nat Bot (IX) 2 167 1905

A variabilis Gasperini in Atti Soc Toscana Nat Sci Pisa Mon 8 fasc 2 326 1887

A pseudo flavus Saito in Centralb Bakt etc 2 Abt 18 No 1/2 p 34 figs 15-18 1907 or its synonym *S pseudo flava* (Saito) Sacc in Syll 22 1260 1913

A siebenmanni Constantin and Lucet in Ann Sci Nat Bot (IX) 2 162 1905 is a bibliographic species based upon an organism isolated from the human ear and diagnosed by Siebenmann (Zeitsch f Ohrenheilk 12 1883) as *A flavus* The describers regarded it as a separate species based only upon the description given by Siebenmann

A gymnosardae Yukawa in *Jour Col Imp Univ Tokoyo* 1 36? Pl 18 figs 1 7 1911 A member of the *A flavus-oryzae* group with measurements intermediate between more typical representatives of the two species

A thomii Graff nomen nudum a heavy sclerotium producing strain distributed by Graff but never described No diagnostic basis for the name was presented

A pollinis Howard in Am Bee Jour 36 577-578 1896 was discussed as an organism causing pickled brood and bee paralysis (See also idem 38 530-531 1898) Turesson (Svensk Bot Tidskr 11 30 1917) decided the mold was *A flavus*

Aspergillus parasiticus Speare in Hawaiian Sugar Planters Exp Sta Path and Physiol Ser Bul 12 p 38 pl 3-4 1912 See Thom and Church The Aspergilli, p 203 1926

Colonies on Czapek s solution agar with sucrose spreading rapidly forming a surface growth of crowded conidiophores with very few sterile

hyphae (fig 73 A) in deeper yellow green shades near ivy green (Ridgway Pl XXXI) reverse uncolored or yellowish Conidial heads radiate abundantly produced and giving color to the colony Conidiophores given by Speare as 300 to 700μ long commonly under 400μ with walls colorless prominently rough or pitted enlarging from 3μ at the foot up to 10 to 12μ and passing into vesicles up to 35μ in diameter (fig 73 B) Sterigmata in one series 7 to 9μ by 2 5 to 3μ closely packed over the vesicular surface yellow Conidia pyriform to globose very rough 4 to 5μ occasionally 6μ in long axis green No sclerotia or perithecia reported

Described as parasitic upon the mealy bug of sugar cane (*Pseudococcus calceolariae* Mask) in Hawaii Type culture NRRL No 502 (Thom No 3509) received from Speare Cultures with the same morphology were isolated from infected mealy bugs from Demerara by Thom and in Louisiana by Kopeloff Johnston working in Puerto Rico considered that he had proved infectivity to be a strain function among organisms of the *A flavus* series rather than associated with morphology Blochwitz (Ann Mycol 32(1/2) 86 1934) has called another nearly allied form *A flavus* var *viridis* but gives no adequate data for separation Cultures with these characters are occasionally obtained from sources not known to be associated with disease of insects Shih has likewise described from China as *Aspergillus chungii* (Lingnan Sci Jour 16(3) 378 1933) a strain which apparently duplicates *A parasiticus*

Aspergillus effusus Tiraboschi in Ann di Bot (Rome) 7 16 fasc 1 1908 See also Thom and Church in Am Jour Bot 8 109–110 1921 and Thom and Church in The Aspergilli p 208 1926

Colonies on Czapek s solution agar rapidly and broadly spreading floccose or piled cottony white (fig 73 C) becoming dirty yellowish or in restricted areas pale greenish yellow then passing over into dull buff or tan shades as heads mature reverse and agar yellowish Conidial heads usually more or less columnar mostly small a few of them fairly large many of them upon short conidiophores (fig 71 B_3 and 73 E) often less than 100μ long and 5 to 10μ in diameter arising from the trailing floccose hyphae quickly losing their yellow green color Conidiophores with walls pitted or roughened sometimes bearing granules (produced by drying droplets of exuded fluid) Vesicles mostly under 20μ in diameter (fig 71 B_2) Sterigmata in one series in small heads in either one or two series in large heads (fig 71 B_1) approximating the *A flavus* type Conidia pyriform to globose varying from 3 by 4μ to 5 by 7μ No sclerotia or perithecia reported The species was described originally from rotten corn (*Zea Mays*)

Culture description as given centers around culture NRRL No 506 (Thom No 130) isolated by Dr B F Lutman Burlington Vermont Other

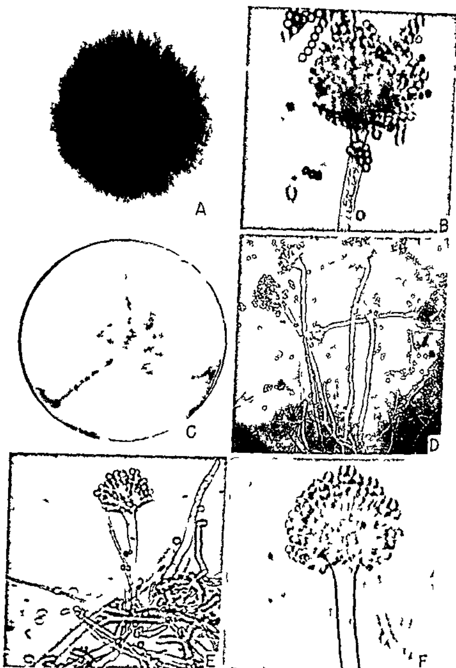


FIG 73 *A* and *B*, *Aspergillus parasiticus* NRRL No 465 1 Colony on Czapek's solution agar showing heavy production of short stalked conidial structures 10 days *B* Single conidial head showing single series of sterigmata and roughened conidiophore × 500 *C–F* *Aspergillus effusus* NRRL No 506 *C* Colony on Czapek's solution agar showing characteristic floccose habit and light sporulation 10 days *D* Conidial structures arising from aerial hyphae characteristic of species × 165 *E* Single short stalked fruiting structure arising from aerial hyphae × 300 *F* Conidial head showing sterigmata mostly in two series roughened surface of conidiophore not apparent × 500

strains studied include isolations from cornmeal from Indiana and from mealy bugs in Puerto Rico Superficially this species bears little relation to *A flavus* Detailed microscopic examination of heads and spores however, reveals true and close relationships Selective transfer from original colonies permitted changes in the predominance of the sterile areas over the fruiting areas without changing the general habit or nature of the colony Blochwitz (Bot Centralb Beiheft Abt Anat Phys 48 176–182 1931) regarded *A effusus* as a floccose type of *A flavus* This position can be supported but the authors feel that the species should be maintained since it is strikingly different from *A flavus* in its general colony appearance, and since it is repeatedly although infrequently isolated from nature

Whether *Sterigmatocystis lutea* Bainier (Bul Soc Bot France 27 27 1880) was one of these can only be guessed from culture NRRL No 508 (Thom No 4640 473) received from the Bainier collection under this name The strain is close to *A effusus* Bainier did not claim identity with *S lutea* van Tieghem (Bul Soc Bot France 24 103 1877) which was entirely undescribed

Aspergillus jeanselmei Ota in Ann de Parasit 1(2) 137–146 1923 as received from Baarn in 1939 (NRRL No 507 Thom No 5665) represented a member of the *A flavus* series with close affinities to *A effusus*

Occurrence

Members of the *A flavus-oryzae* group are among the most abundant of all the Aspergilli They are world wide in distribution and are omnivorous in the substrata upon which they are able to grow and develop They have been isolated from the widest variety of sources including the fermentation industries of the Orient grains and cereal products from different parts of the United States various types of forage egg noodles bread and other bakery products leather goods dried dates cured meats dairy products nut meats soy sauce home-canned fruits and vegetables textiles paper pulps insects tannin inoculum feces sputum the lung of a bird and from the duodenum of man They are very abundant in soil and have been observed in almost all samples examined They appear to be particularly common in the warm soils from tropical and sub tropical areas They vary greatly in cultural appearance and in the detailed measurements of their fruiting structures and to a limited degree these differences can be correlated with the sources from which they are obtained Soil isolates commonly show conidial heads near yellow green in color which are borne upon comparatively short conidiophores Isolates from the rice and soy fermentations of the Orient often show conidial heads pale yellow green in color that are borne upon long thin walled conidiophores Exceptions to this very general statement are common

Kojic Acid

The ability of members of the *A flavus oryzae* group to produce kojic acid has been recognized for more than three decades It is only within the past fifteen years, however that serious attention has been given to this fermentation Beginning with the work of Challenger Klein and Walker in 1929 and 1931, and continuing with that of May, Herrick Moyer, Ward and Wells in 1931 and 1932 the proper nutrients and cultural conditions necessary for its production were defined Subsequent contributions have been made by Kluyver and Perquin (1933) and by Barham and Smits (1936) In all of the early reports the responsible cultures were cited as *A oryzae* whereas in more recent ones the cultures employed have generally been identified as *Aspergillus flavus* It is of interest to note that the strain studied by May and associates (NRRL No 484 Thom No 3538) was a thoroughly typical *A flavus* when first isolated by Thom in 1914 but during the long period that it has been maintained in artificial culture it has gradually changed until today it more nearly resembles *A oryzae* in its general habit and coloration (fig 72 C) Its capacity to produce kojic acid remains undiminished however The culture employed by Barham (NRRL No 625) likewise fails to satisfy the typical cultural picture of *A flavus* although it is discussed under this name In contrast to these cultures other strains belonging to this group have been under continuous laboratory cultivation for more than 30 years without apparent change in appearance or behavior The above and additional references to the kojic acid fermentation are presented in the Topical Bibliography, pp 297–298

Enzymes

Members of the *A flavus-oryzae* group produce diastatic and proteolytic enzymes abundantly For this reason they have been much studied and an extensive literature regarding mold enzymes has developed around the use of these fungi In large measure the alcoholic and soy food industries of the Far East are based upon these molds and their enzymes In the production of alcoholic beverages the diastatic enzymes produced by an Aspergillus (regularly identified as *A oryzae*) are employed to hydrolyze the rice starch Alcohol is then produced from the resultant sugars by the addition of a fermentative yeast In the soy industries closely related molds or even the same strains are used as a source of proteolytic enzymes In 1894 Takamine secured a series of U S patents covering the production of diastatic enzymes and the making of alcoholic liquors (see Topical Bibliography p 302) Subsequent to this other investigators mostly Japanese published a number of papers in this field Oshima in 1922 and 1928 reported on the production of protease by members of the *A flavus*

oryzae group Today considerable quantities of diastatic enzymes proteolytic enzymes and mixed diastatic and proteolytic preparations are being manufactured from these molds for use in the textile and tanning industries particularly Within recent years considerable attention has been given to 'moldy bran (bran seeded with selected strains of *A oryzae*) as a possible substitute for malted barley as a saccharifying agent in the production of industrial alcohol (Underkofler Fulmer and Schoene 1939 Schoene Fulmer and Underkofler 1940 Hao 1942 Hao Fulmer and Underkofler 1943 Christensen 1943)

References to papers dealing with the production of enzymes by molds belonging to this group are presented in the Topical Bibliography pp 302–304 No attempt has been made to present a complete bibliography of the subject, but it is believed that sufficient citations are listed to introduce the reader to the extensive literature of the field

Pathogenesis (See Topical Bibliography pp 307–310)

Pathogenesis has been occasionally reported for strains identified as members of the *A flavus* series Observations reporting their presence in the external ear go back to Siebenmann (1882) Ota described *A jeanselmei* as a parasite of human nails in Paris in 1923 Bereston and Keil (1941) described a case of infected nails in which the strain as seen by us proved to be a variant of *A flavus* There are ample records to show an occasional infection of the human being There are no data as to the route of infection and the question whether the parasite is a primary or a secondary (wound) parasite stands unanswered The constant presence of members of this group in every human environment together with a lack of evidence of *ability to penetrate sound human tissue* leaves some doubt—*not as to its* ability to grow when once established but whether it can actually break and enter as a direct agent of disease *Aspergillus flavus* is commonly isolated from sputum In birds cases of lung involvement have been reported There is no question but that the mold can persist for reasonable periods of time within the animal body *A flavus* is one of the more common air borne molds and occasional allergic reactions are attributed to it although instances where it is the sole responsible agent are not known to have been reported

Antibiosis

The production of antibacterial substances by strains of *Aspergillus flavus* has been observed by a number of workers during the past five years White (1940) reported a culture of *A flavus* (found by the writers to be a somewhat atypical strain) to produce some substance which was definitely bactericidal against some gram negative and some gram positive bacteria

A more detailed study of this strain was subsequently made by White and Hill (1943) and the name "aspergillic acid" was assigned to the active substance The compound shows a comparatively high toxicity to laboratory animals Utilizing the White strain, Jones Rake, and Hamre (1943) have made additional studies on the biological properties of aspergillic acid In the meantime Glister (1941), at Oxford University reported the production of an antibacterial substance effective against gram negative and gram positive bacteria by a different strain of *A flavus* (found by the writers to be wholly typical of the species) He noted the possibility of relationship between the substance with which he was working and that earlier reported by White

Following the work of Jones Rake, and Hamre (1943), McKee and MacPhillamy (1943) succeeded in demonstrating the production of a second and entirely different antibacterial substance In certain chemical properties and in its action on bacteria this was found to resemble penicillin very closely, but actual identity was not proved In a subsequent and more detailed report, McKee, Rake, and Houck (1944) defined more exactly its bactericidal action against various gram negative and gram positive bacteria and proved additional evidence of its penicillin like characters They designated the substance "*flavicidin*"

Concurrent with this work Bush and Goth (1943a and 1943b), working at Vanderbilt University succeeded in demonstrating the production of an antibacterial substance from still another strain of *A flavus* which was strongly active against *Staphylococcus* and other gram positive forms but comparatively inactive against gram negative forms belonging to the *E coli* group The substance was termed 'flavicin Identity with flavicidin and with penicillin is possible but has not yet been proved Cook and Lacey (1944) report the production of appreciable amounts of an antibiotic substance from a strain of *A parasiticus* This was provisionally designated "parasiticin", and its similarity to penicillin was noted Identity with flavicin (Bush and Goth) and flavicidin (McKee Rake and Houck) was suggested Since *A parasiticus* is so closely related to *A flavus* it would seem probable that an antibiotic produced by it would be similar to that produced by the latter species under the same conditions

Waksman and Bugie (1943) investigated a large number of strains belonging to the *A flavus-oryzae* group They found strains of *A oryzae* to show little activity whereas strains of *A flavus* showed increased but varying amounts Yields were markedly influenced by various nutritional and environmental factors Two types of antibacterial substances were observed aspergillic acid and a substance similar to if not identical with penicillin When grown in submerged culture one strain was found to produce amounts comparable to the best strains of *Penicillium notatum* tested Unfortunately yields were not quantitatively determined

CHAPTER XXI
THE ASPERGILLUS OCHRACEUS GROUP

Outstanding Characters

Conidial heads ranging from sulphur yellow to varying shades of ochraceous depending upon the species and strain showing a greenish tint only in the single species *A sparsus* heads globose or radiate with conidial chains commonly adhering into divergent columns

Conidiophores normally showing shades of yellow in the outer layers of the wall which is rough or pitted usually prominently but occasionally reduced to traces which are seen most readily in dry mounts

Sterigmata in two series with the primary often quite large and septate

Conidia in some series thin walled and smooth in others showing definitely double walls more or less roughened or echinulate

Sclerotia present in most species and strains often dominating the cultures in others entirely lacking When present ranging in color from cream or buff through pink and orange shades to purplish vinaceous

Molds belonging to this group are common wherever organic matter is decomposing under natural conditions In spite of great variation in superficial appearance length of conidiophore size of heads intensity and shades of color and sclerotium production they fit together into a great natural group of related forms Extreme variants in the several series can be easily considered separate species and have been so described but collections of great numbers of such forms present so many gradations that identification by description becomes doubtful if not impossible Allocation to series centered upon some described species gives a practical method of grouping together closely related members of the great aggregate

The name ochraceus derived from the pigment ocher and attached to the most abundant series in the group is an old mycological usage which was more or less definitely followed in Saccardo s use of it for a plaque in his Chromotaxia Ridgway analyzed the colors more exactly dropped the term ochraceus but included a plaque near to it as Pl XV Col 15 YO Ochraceous Buff The strains of this group however mostly show colors closer to the yellower tints in Ridgway s Plate XXX column 19 It is important that relationship with the great group shall be quickly grasped whether exact identity with particular strains already known is claimed or not

Group characterization The colony appearance of this group of Aspergilli varies greatly with the presence or absence of sclerotia Some species

low globose heads Sclerotia were not reported In our experience however they are seen not infrequently in cultures which otherwise fit the description of *A sulphureus* as presented In the sub series, represented by *A quercinus* (Bainier, Thom and Church) sclerotia are very abundant and heads comparatively few and scattered among the sclerotia In addition to these two names which roughly designate sections or series of isolates as we find them every gradation between these extremes may be anticipated

The species is fairly common in soils, on cereal grains and upon decaying vegetation

Several species have been described as having conidial heads sulphur yellow but doubtfully separable from *A sulphureus*

S ochroleuca Spegazzini in Myc Argent V p 434 in Anal Mus Nac Buenos Aires Ser 3 T 13 1911

S auricoma Gueguen in Bul Soc Myc France 15 171-187 figs 1-48 1899 This appears to have been a member of the series with primary sterigmata proliferating abundantly to form little conidiophores and secondary heads thus giving the head the appearance of bearing yellow hair Such proliferation occasionally occurs in many species but has not again been found in this group

S vitellina Ridley in Jour Bot (London) 34 152, pl 357, figs 14-16 1896 is described as producing bundles or coremia composed of partially adherent conidiophores The organism does not appear to have been cultivated and has not since been reported

Aspergillus quercinus (Bainier) Thom and Church in The Aspergilli p 186 1926

Synonym *S quercina* Bainier in Bull Soc Bot France 28 78 1881
See also Sartory Etude biologique du *Sterigmatocystis quercina* Bainier in Bul Soc Myc France 26 349 1910

Colonies upon Czapek s solution agar spreading broadly characterized by the presence of an aerial white mycelium and the abundant production of sclerotia over the whole area (Pl VII E and fig 74 A) or in sectors or variously distributed the mass changing from yellow to orange yellow and finally to rufous or brick red shades with the ripening of the sclerotia reverse in shades of yellowish-orange Conidial heads in yellow tints near sulphureus scattered among the sclerotia or occurring in long stalked groups in the dryer areas of the culture tube or plate mostly up to 200μ in diameter but occasionally larger up to 300 or even 400μ (fig 74 C) Conidiophores with walls mostly pale yellow especially in the outer layer, pitted (fig 74 D) occasionally with abundant granules about 2μ in thickness varying from short and inconspicuous in crowded sclerotial areas to very long tufts in dryer areas up to 2 or even several millimeters by 10 to 20μ Vesicles colorless crushing easily 35 to 45μ in diameter fertile over

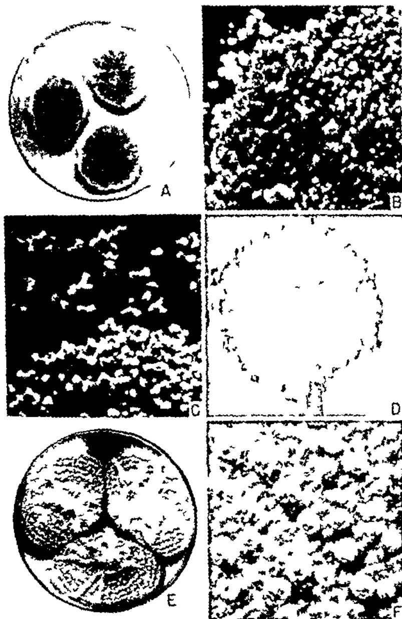


Fig 74 *A–D* *Aspergillus quercinus* NRRL No 394 *A* Colonies on Czapek's solution agar 10 days predominantly sclerotial but with localized areas of heavy conidial production in central areas *B* Marginal area of the same colony showing very abundant sclerotia and scattered conidial heads in lower right hand corner *C* Conidial heads on hay infusion agar plate × 18 *D* Single head showing globose character crowded sterigmata and roughened conidiophore *E* *Aspergillus sclerotiorum* on Czapek's solution agar 10 days *F* Portion of the same enlarged to show abundant large sclerotia and scattered comparatively small heads × 6

color and appearance (Pl VII F and fig 75 A) reverse ranges from colorless through orange to purplish shades

Conidial heads when large mostly globose or variously splitting into masses of conidial chains (fig 75 C) variously colored from very pale to deep ochraceous shades Conidiophores vary greatly in length and diameter

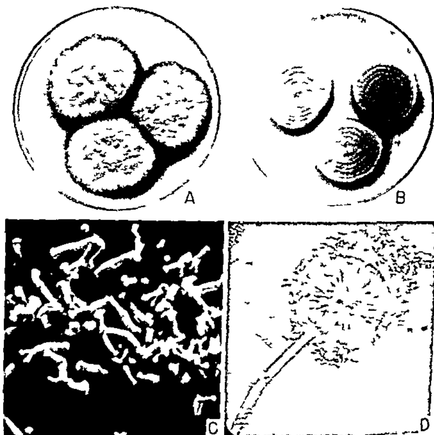


FIG 75 *Aspergillus ochraceus* series *A* and *B* *A ochraceus* NRRL No 398 and No 408 respectively, growing on Czapek s solution agar at room temperature 10 days *C* Mature heads of strain No 398 the tendency to split into divergent columns in age is characteristic *D* Photomicrograph of a single head showing globose vesicle sterigmata in two series and coarsely roughened conidiophore × 325

in the various strains but typically show a yellow color in the outer layers of the thick wall which shows characteristic pitting or roughening (fig 75 D) Vesicles mostly globose or somewhat elliptical and fertile over the whole surface (fig 75 D) Sterigmata in two series primary varying from small to very large commonly 15 to 30μ in length secondary fairly uniform

commonly 7 to 10μ by 1.5 to 2.5μ. Conidia globose, subglobose or somewhat elliptical, more or less rough or echinulate, ranging from 3.5 to 5.0μ in long axis. No perithecia reported. Sclerotia are present in Wilhelm's material preserved in exsiccati.

Among the great number of isolates belonging to this group a considerable number show approximately the characters as drawn from Wilhelm's description and his exsiccati. In the limited sense then *A. ochraceus* Wilhelm can be interpreted to apply to those ochraceous strains which bear echinulate conidia from 3.5 to 5.0μ in diameter and produce sclerotia. Among the strains included almost all are found to produce colonies of consistent aspect in continuous culture.

Aspergillus elegans Gasperini in Atti Soc. Toscana Sci. Nat. Pisa Mem. 8: 328, fasc. 2, 1887.

Synonym: *S. elegans* (Gasp.) Sacc. in Syll. 10: 525.

This species by description differs little from *A. ochraceus* Wilhelm except for the absence of sclerotia and conidia which do not exceed 3.5μ in diameter. Thom and Church did not recognize it as a valid species in 1926 and one may be certain that no sharp line can be drawn separating *A. elegans* from *A. ochraceus*. Nevertheless, forms producing conidia consistently less than 3.5μ are commonly encountered among miscellaneous isolations from nature and there is an argument for retaining a species to include such forms. The following species diagnosis taken from Saccardo (10: 525) was presented by Thom and Church (1926).

Mycelium white, stalks continuous, unbranched, hyaline then pale ochraceous, 1 to 6 mm. long by 5 to 12μ in diameter, delicately studded with drops; vesicle up to 70μ diameter, radiate, entirely covered with sterigmata; sterigmata primary 4 to 26μ long, secondary 7 to 14μ long by 1 to 2μ; conidia ochraceous, elliptical to globose, up to 3 to 3.5μ, with wall very delicately verruculose; sclerotia not found.

Various other species have been described which are obviously closely related and belong to the *A. ochraceus* series. A partial list would include:

S. helva Bainier in Bull. Soc. Bot. France 28: 78, 1881. Thom's culture No. 4640.476, received under this name, came from the Bainier collection.

A. alutaceus Berkeley and Curtis (in Grevillea 3, No. 25, p. 108, 1875) was the name proposed for a mold found upon corn. The specimen is preserved as No. 3793 in Curtis' Herbarium now in the Cryptogamic Herbarium of Harvard University. The specimen shows that this species was probably a strain of *A. ochraceus*.

A. ochraceus var. *microspora* Tiraboschi (in Ann. di Bot. [Rome] 7: 14, 1908) represents a strain in which all measurements were reported as reduced.

A. rehmii Zukal. A culture from Dr. Westerdijk received under this name is also a member of this series.

Certain large-spored species of doubtful validity and relationship have been described that are believed to represent members of the *A ochraceus* series Although obviously rare, these species were described with sufficiently distinctive characters to warrant their retention With continued search, it is possible that they may be reisolated

Aspergillus delacroixii (Sacc) Thom and Church, in The Aspergilli, p 190 1926

Synonym *S ochracea* Delacroix in Bull Soc Myc France 7 109 Pl VII, fig f 1891 (Delacroix failed to recognize the previous use of the specific name)
S delacroixii Sacc in Sacc 10 527

This species is reported as having conidiophores pale yellow and rough 500 to 1000μ in length, vesicle globose thick walled, punctate yellow, sterigmata in two series primary 39μ by 12μ secondary (from Delacroix s figures) about 8 to 10μ by 2 to 3μ conidia globose finely roughened 7 to 8μ in diameter *The yellow and roughened conidiophores ally this species with* the *A ochraceus* group, hence would justify the description if a form with such large conidia should be found again Otherwise it is possible that some old material of a strain of *A oryzae* might have furnished the type

Aspergillus butyracea (Bainier) *n* comb

Synonym *S butyracea* Bainier in Bull Soc Bot France 27 29 1880
Specimen attributed to Bainier in C Roumeguere s Fungi Gallici Exsiccati No 995

This is described as a large spored strain belonging in this group The specimen showed a black Aspergillus as well as an ochraceous form with spores up to about 6μ and rough Colonies butter yellow including *conidiophores heads and conidia conidiophores* yellow in mounts finely punctate or pitted 13 to 16μ in diameter sterigmata primary up to 25μ secondary 10 to 12μ in length conidia described as smooth 5 2μ but those *found in the material were rough and up to* 6 3μ It is entirely possible that this species may be found again but is has not been reported since Bainier described it

A penicillopsis (Henngs) Racib P Hennings Synonym *Stilbothamnium penicillopsis P Henn and E Nym* described in Fungi Monsunenses (Warburg O Monsunia Bd I p 37 1900 Leipzig) exsiccati of type in Pathological Collections U S Dept Agr Bureau of Plant Industry as Raciborski no 87 in Crypt Parasiticae Java

This material shows an Aspergillus with the color and general appearance of *A ochraceus* but of *gigantic proportions Measurements* as follows

Conidiophore 50 to 70μ in diameter 10 mm or more long with walls 7 to 12μ thick Surface ragged and more or less pitted Vesicle up to 175μ in diameter with walls 7μ thick marked with a deep pit for each sterigma Sterigmata primary 50 to 90μ at times 120 by 8 to 10μ at the outer end sometimes with one cross wall secondary 15 to 25μ by 3 to 4μ clustered on the apex of the primary Occasionally with a sterile cell interposed between secondaries and primaries Conidia 8 to 12μ by 5 to 8μ elliptical pitted yellowish

Gigantic forms such as this occasionally appear under field conditions hence reach fungus herbaria Whether these are really different from some of the usual species can only be determined by collection and laboratory cultivation Until such study has been made such forms as *A penicillopsis* must be questioned

Aspergillus ostianus Wehmer in Bot Centralb 80 449–461 1899 also Monogr pps 117–119 Taf II No 1 1899–1901

Colonies upon Czapek s solution agar growing fairly well producing a surface growth of crowded conidiophores and conidial heads in yellowish to ochraceous shades passing to shades of cinnamon in very old cultures with reddish brown colors in reverse (Wehmer reported rusty yellow, pale to deep brownish yellow to cinnamon) Conidial heads globose up to 200μ in diameter Conidiophores yellow coarsely roughened mostly 500 to 700μ long in crowded areas becoming 1 to 2 mm in margins of old colonies and usually 7 to 10μ in diameter with walls heavy up to 2 or 2 5μ in thickness Vesicles commonly globose about 40μ in diameter occasionally much larger up to 70μ in growing heads thin walled colorless crushing easily leaving the funnel like yellow tip of the conidiophore open Sterigmata in two series primary from 15 to 20μ long in smaller heads to 35μ by about 8μ at the tip in large heads secondary 10 to 13μ by about 3μ Conidia commonly 3 to 4μ or even 5μ in long axis varying from pyriform to elliptical or at times subglobose rough Sclerotia occasionally present but not conspicuous

This diagnosis was based upon Wehmer s description and is reasonably well represented by strain NRRL No 420 (Thom No 4724 35) received from Raistrick in 1924 and reported by him to have come from Westerdijk as Wehmer s original strain With conspicuous appearances suggesting relationship to the *A ochraceus* group the shape and ultimate color of the ripe spores suggest a border line position between *A ochraceus* and *A tamarii*

Aspergillus sparsus Raper and Thom in Mycologia 36 572–574 fig 6 1944

Colonies upon Czapek s solution agar at room temperature spreading broadly dull grayish brown in color at first largely submerged but later

developing limited aerial growth, giving rise to widely scattered er conidial structures (fig 76 A) characterized by dull greenish tan heads affecting the color of the colony as a whole, reverse in brown shades or none Colonies upon hay infusion agar spreading broadly, almost who submerged giving rise to scattered but conspicuous conidial structu (fig 76 B) often in definite concentric zones heads globose radiate, in oliv

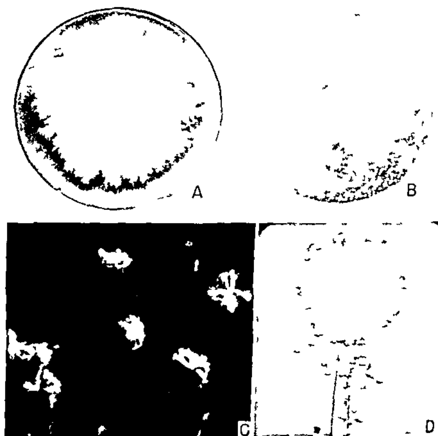


Fig 76 *Aspergillus sparsus* *A* and *B* Colonies on malt extract and hay infusion agars respectively 2 weeks note scattered heads in *B* and almost complete absence in *A* *C* Conidial heads × 18 *D* Single conidial head showing globose vesicle and roughened conidiophore × 425

buff shades (Ridgway, Pl XL) Colonies upon malt extract agar spreading irregularly floccose 1 to 2 mm deep cream buff in color conidial structures very few in number, heads globose radiate dull olive yellow in color (Ridgway, Pl XL) Conidial structures generally few in number never abundant erect arising from a submerged mycelium heads typically globose becoming more or less radiate in age (fig 76 C) mostly 200 to 250µ

in diameter occasionally as much as 500μ pale olive buff to olive buff (Ridgway Pl XL) upon Czapek and hay infusion agars to pale olivine or olivine (Ridgway Pl XXXII) upon malt extract agar Conidiophores straight mostly 1 to 1½ mm in length by 10 to 12μ in diameter approximately uniform in diameter throughout, wall 1 2 to 1 5μ in thickness conspicuously echinulate typically arising from a foot cell enmeshed in a network of feeder hyphae often tapering abruptly in the region immediately beneath the vesicle Vesicle comparatively thin walled globose (fig 76 D) mostly 40 to 50μ in diameter occasionally larger or smaller bearing sterigmata over the entire surface Sterigmata in two series primaries crowded comparatively short and stout commonly 8 to 10μ by 3 to 5μ secondaries 6 to 8μ by 2 5 to 3 5μ Conidia pale yellowish in mass individually showing slight coloration subglobose to slightly elliptical very finely roughened mostly 3 to 3 5μ in long axis

Type culture NRRL No 1933 was isolated in February 1943 from soil collected in La Lima Honduras by Dr L A Underkofler A second strain which duplicates the type almost exactly was subsequently isolated from soil collected in Bexar County Texas by Sister Mary Clare of Our Lady of the Lake College San Antonio Texas

The correct position of this species within the genus Aspergillus is open to question The presence of a colored coarsely roughened conidiophore indicates close relationship with *Aspergillus ochraceus* This is likewise supported by the globose vesicle and head although these characters are typical of other groups as well The scarcity of fruiting structures upon all media and more particularly the greenish tint of the spore masses however tend to set it apart from the common representatives of this great group The general habit of the colonies together with the paucity of conidial structures is strongly suggestive of *Aspergillus alliaceus* but this latter species does not show any trace of greenish color in its conidial heads it does possess smooth colorless conidiophores and upon ordinary culture media regularly produces an abundance of black sclerotia which very often dominates and characterizes the culture In the color and character of its conidial heads *A sparsus* is somewhat suggestive of George Smith s new species *A avenaceus* (Trans Bul Mycol Soc 25 24-27 Pl I 1943) but it differs from this as it does from *A alliaceus* in possessing rough conidiophores and in its failure to produce sclerotia Until additional related forms are isolated we believe it best to consider this species as a member of the *A ochraceus* group realizing that it does not entirely fit this placement as the group has hitherto been considered

Occurrence and Economic Importance

Members of the *A ochraceus* group are widely distributed in nature and can be obtained from a variety of sources They are especially common

in soils and have been isolated from samples collected in many parts of the world Within the group strains approximating the species *A ochraceus* are by far the most abundant, although heavy sclerotium producing strains approximating *A quercinus* are not infrequently encountered They are a common component of the microflora of decaying vegetation, but there is little evidence that they play a very active role in processes of decomposition

Huber (1933) found a member of the group *A sclerotiorum*, capable of rotting apples and pears Members of the group are commonly found in musty or moldy cereal grains, but are not as characteristic of this substratum as is *Aspergillus candidus* and members of the *A glaucus* group In at least one instance *A ochraceus* was reported as a human pathogen (Ceni 1905)

In the Orient, *A ochraceus* and allied species constitute a portion of the mold flora characteristically found on "Katsuobushi' and other fermented preparations made from fish (Yukawa 1911) *Aspergillus melleus* Yukawa was isolated from such material Because of the mixture of forms present, including members of the *A glaucus* and *A flavus oryzae* groups it is probably incorrect to say that any particular species or group of species is responsible for this fermentation (See also Hanzawa 1911)

A ochraceus has been used to bring about desired changes in the flavor of coffee and its use is covered by U S Patent No 1,313 209 Samples of the fermenting coffee showed the organism used to be a strain of *A ochraceus* indistinguishable from Wilhelm s species Whereas *A niger*, *A tamarii* and *A flavus* were also capable of developing in the fermenting coffee *A ochraceus* alone of the species tried gave a satisfactory flavor

As a whole, the *A ochraceus* group constitutes a very abundant but little studied, group of molds Whether the scarcity of published reports regarding biochemical activities indicates an absence of such, or whether it merely reflects a limited amount of investigation can at present only be guessed

PART III

REFERENCE MATERIAL

CHAPTER XXII

TOPICAL BIBLIOGRAPHY

In preparing this manual the writers have considered it inadvisable to attempt to discuss the biochemical activities of the Aspergilli since this subject is book length in itself Furthermore the aim of the manual is to provide the mycologist and microbiologist with a means of identifying and interpreting Aspergilli as they are isolated from nature and to furnish the chemist working with molds with a guide which will enable him to maintain industrially important cultures in an optimum condition Nevertheless because of the increasing importance of the Aspergilli as agents responsible for industrial fermentations as subjects for physiological and biochemical investigations and now as possible sources of various antibiotics it has seemed advisable to present a topical bibliography dealing with these and related subjects We have not attempted to present a complete bibliography but rather to present a sufficient number of references to provide the investigator and student with an entrée to the literature of particular fields An attempt has been made to choose the more important papers for citation Believing that more recent contributions will generally be of the greatest interest and value to the user this topical bibliography is presented in chronological rather than alphabetical order A list of subjects under which references are presented follows

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Yamamoto Y 1927 Ueber drei Stämme von *Aspergillus wentii* (Japanisch) Jour Soc Agr Forest Sapporo 19 123–40

Yuill Edward 1939 Two new Aspergillus mutants Jour Bot (London) 174–175 illus

—— and Yuill John L 1938 *Cladosarum olivaceum* a new Hyphomycete Brit Mycol Soc Trans 22 194–?00 illus

Yukawa M 1911 Zwei neue Aspergillusarten aus Katsuobushi Jour Coll Agr Tokyo 1 357–366

Zikes H 1922 Uber die Perithecienbildung bei *A oryzae* Centralb f Bakt etc 2 Abt 56 339–343

Chapter XXIV

CHECK LIST OF SPECIES AND GENERA

GENERIC NAMES FOUND APPLIED TO ASPERGILLI

Allescheria G Pim in Proc R I Acad 1883 and Jour Bot 1883 p 234 also Sacc Syll 18 No 5 16 1906 4 *Sapucaya* represents a monotypic genus found upon putrifying *Lecythis Sapucajo* From the description it was some member of the *A niger* group Saccardo s diagnosis only seen 9

Ascophora *Ascophora nigrans* was vaguely assigned mostly to Mucors but appears to have been used by Raulin s group studying the tannic acid fermentation until Van Tieghem described *A niger* 9

Aspergillopsis Sopp in Videnskaps selskapets Skr I Mat Naturv Kl No 11 pp 201 204 Taf XX fig 149 1912 Sopp probably had some members of the *A ustus* group 9

Aspergillopsis Spegazzini in An Mus Nat Buenos Aires Ser 3 13 434 1911 The black spored Aspergilli (essentially the *A niger* group) were regarded as dematiaceous and assigned to a new genus in that group Spegazzini s genus has not been accepted 8

Aspergillus Micheli in Nova Plantarum Genera p 212 Pl 91 1729 Compare Link in Obs p 16 1809 and Corda in Icones Fungorum 4 31 Tab VII fig 94 1840 6

Subgenus—*Microaspergillus* Wehmer in Monogr 1901 (Mémoires de la Société de Physique et d Histoire Naturelle de Genève Tome XXXIII Seconde Parte pp 1 157 1899–1901) 16

Subgenus—*Macroaspergillus* Wehmer in Monogr 1901 The subgenera are not accepted here 16

Cladosarum Yuill and Yuill in Trans Brit Myc Soc 22 194–200 Fls 11–13 1938 Regarded as a laboratory mutant not encountered in nature In *A niger* group See 73

Dimargaris van Tieghem in Ann Sci Nat 6 Ser 1 54 18 5 The name *Dimargaris* sp has been seen upon living cultures in one of the great laboratory collections These cultures were obtained from dog and squirrel dung The organism so labeled belonged to the *Aspergillus candidus* group The genus *Dimargaris* (type *D crystalligena* v Tieghem) is regarded by Fitzpatrick as closely related to *Dispira* and as a synonym of *Dispira* by Zycha (Kryptogamenflora der Mark Brandenburg Pilze II Mucorineae Band VIa 1 264 114 figs 1935) In any case it is only reported as a parasite of the mycelia of the Mucorineae hence certainly was not an *Aspergillus* 9

Diplostephanus Langeron in Compt Rend Soc Biol Paris 87 343–345 1922 This genus is proposed by Langeron to include ascosporic Aspergilli (*Sterigmatocystis*) with two series of

sterigmata *A nidulans* Eidam is named as type The proposal is rejected here 8

Emericella Berkeley and Broome in Introd Crypt Bot pp 340–341 fig 76 1857 See also Patouillard in Bul Soc Myc France 7 43–49 pl 4 figs 6–12 1891 Based upon *E variecolor* Syn *A variecolor* q v 163

Euaspergillus Ludwig in Lehrbuch der niederen Kryptogamen p 258 Stuttgart 1892 The genus was proposed to cover the sclerotium producing Aspergilli such as *A niger* *A flavus* *A ochraceus* The name has not been accepted 8

Eurotium Link in Obs p 31 Taf 2 fig 44 1809 Link described *Aspergillus glaucus* as a conidial mold and *Eurotium herbariorum* for the ascosporic form supposing them to be different fungi His genus *Eurotium* has been widely accepted but it is rejected here for reasons discussed elsewhere Forms listed as *Eurotium* but having by description black perithecia are excluded No species with aspergilloid conidial structures has black (Dematiaceous) perithecia 7

In engaea Borzi in Jahrb Wiss Bot (Pringsheim) 16 450–463 Pl 19 20 (1884) 1885 Type sp *I erythrospora* Borzi Syn *A variecolor* q v 165

Mucor used by Wiggers (Wichers) in Primitiae Florae Hol aticae 1780 (Republished in facsimile Edition No 23 by W Junk in 1925) Mucor herbariorum sessilis luteus is given as No 1158 7

Rhodocephalus Corda in Icones I 21 1839 and the figures given for *R aureus* in Icones III Taf II fig 33 suggest *A terreus*

Sartorya Vuillemin in Compt Rend Acad Sci (Paris) 184 136–317 1927 Inadequately described as an ascosporic phase of *A fumigatus* It was not distributed in culture 9

Sterigmatocystis Cramer in Viertel jahresschrift der Naturforchungen Gesellschaft Zurich 4 325 1859 Type *A antacustica* Cramer Cramer proposed to include in his new genus all aspergilloid species showing both primary and secondary sterigmata *S nigra (A niger)* was the organism under discussion hence the type species Many mycologists have agreed with Cramer others such as Fischer Wehmer and Thom have rejected *Sterigmatocystis* 8

CHECK LIST OF NAMED SPECIES

S acini uvae Caballero in Boletin R Soc Esp Hist Nat 28 429 1928 229
Syn *A carbonarius* fide Blochwitz or *A pulchellus* fide Mosseray 229

S aerea Bainier in Bul Soc Bot France 28 78 1881 *A wentii* group 249

A africanus Dur et M in Fl Alg I P 342 1849 Not an Aspergillus

A agens This name is cited by Lindt in Arch Exp Path Pharm 25 265 1889 as taken from Saccardo s Sylloge Search for this reference leads to the conclusion that in this citation *A Hageni* was made to read *A agens*

S alba Bainier in Bul Soc Bot France 27 30 1880 Some member of *A candidus* group 211

S alba cyanogena Biourge listed among cultures in the Biourge Collection 1939

S alba lutea Biourge n m in MS p 9 among the niveus series

A alba roseus attached to a culture in the Bainier Collection and by inference in the Biourge Collection as near *A niveus* Blochwitz Thom s No 4640 490

S alba sclerotifera Biourge nomen nudum listed in his MS as related to *A okazakii* It produces a violet or blue color in reverse

S alba sulphurea Biourge nomen nudum listed in MS as near *A okazakii* It produces a blue or violet reverse

A albidus Eichelbaum in Verhandlungen d Naturw Ver Hamburg 3 Folge XIV 35 1906
Syn *E albidum* Sacc Syll 22 1254 1913

A albidus Speg A culture under this label distributed by Biourge represents a strain of *A niveo glaucus* q v 135

S albo lutea Bainier in Bull Soc Bot France 27 30 1880 This was a small pale yellow form but no further data were given and it has not since been identified Apparently close to *A carneus*

S albo lutea Sartory Sartory and Meyer cited by Blochwitz in Ann Mycol 31 73 1933 Some member of the *A candidus* group 211

A albo marginatus Biourge in MS p ? as change of name from *Penicillium albo marginatum* figured only in the Penicillium Monograph in La Cellule tome XXXIII 1 fasc fig 129 It was some member of the *A restrictus* series received and studied as Thom No 4733 134a Near *A gracilis* in the *A glaucus* group 139

S albo osea Sartory Sartory and Meyer in Ann Mycol 28 358–359 Pl III figs 1–6 1930 Compare *S albo rosea* in Blochwitz Ann Mycol 31 73 1933 *A carneus* series 202

A albus Wilhelm Inaug Diss Strassburg p 69 1877 Probably *A albus* Haller *S alba* Sacc *Monilia alba* Persoon In *A candidus* group 211

A alliaceus Thom and Church The Aspergilli p 163 1926 See *A wentii* group 244

A alternatus Berkeley in Ann Nat Hist Ser 1 Vol 1 262 1838 Not an Aspergillus

A alutaceus B and C in Grevillea 3 No 25 p 108 1875 In the *A ochraceus* group unidentifiable to strain or series 281

S ambari (alternative spelling *ambaris*) Beauregard in Ann de Micrographie 10 255–278 1 plate 1898 Cited without description in Compt Rend Hebdom des Séances de la Soc de Biol 28 Mai (1898) *A versicolor* group 192

A amoenus Roburg in Hedwigia 70 138–9 1930 Distributed by the Centraal bureau voor Schimmelcultures Baarn Received by us on November 20 1933 and determined as a member of the *A gracilis* series but the description allies it with *A versicolor*

A amstelodami (Mangin) Thom and Church in The Aspergilli p 113 1926 also Thom and Raper U S D A Misc Publ 426 pp 22–26 1941 132
Syn *E amstelodami* Mangin in Sci Nat Bot Ser 9 10 360–361 1909 *A glaucus* group 122
Incorrectly spelled *A amsterodami* in Nakazawa Jour Agr Chem Soc Japan 10(2) 1934
Syn *E repens* var *amstelodami* Vuill in Soc Mycol de France Bul Trimest 36 131 1920
var *alophote* Duche cited by Dodge in His Med Mycol p 630 1935 Ascospore lacks the crest of the species

A *anomalus* Mosseray in La Cellule XLIII 248-249 pl 4 figs 107-112 1934 Member of *A niger* group 234

S *antacustica* Cramer in Vrtljschr Naturf Gesell Zürich Jahrg 4 Heft 4 325 1859 In *A niger* group 239

Syn *A phoenicis* in *A niger* group 222

A *archaeoflavus* Blochwitz in Ann Mycol 31(1/2) 73-83 1933 The type culture (Thom No 250-5346) was too close to *A wentii* for satisfactory separation 247

A *archiflavipes* Blochwitz in Ann Mycol 32(1/2) 84 1934 A form of *A flavipes* characterized by deep brown to red shades of color in the mycelium 181

A *argentinus* Spegazzini in Rev Agr Veter LaPlata p 245 1896 A conidial form in the *A glaucus* group not identifiable

A *argillaceus* was distributed but not described by Biourge It appears to be a non ascosporic strain of the *A repens* series 111

A *atro fuscus* Mosseray in La Cellule XLIII 269-270 pl 4 figs 126-129 1934 Member of *A niger* group 235

A *atropurpureus* Zimmermann in Centbl Bakt [etc] Abt 2 Bd 8 No 5 p 218 1902 *A niger* group 226

A *atropurpureus* Blochwitz in Ann Mycol 32(1/2) 86 1934 Blochwitz proposed this name to cover purple brown series in *A niger* group 225

A *atro ruber* Estienne

Syn *Penicillium roseo-cinnabarinum* Biourge in La Cellule 33 fasc 11 319-321 Pl XVII fig 97 1923

Syn *Physomyces heterosporus* Harz fide Biourge

Syn *S atro rubra* (Estienne) Biourge MS

A *atro violaceus* Mosseray in La Cellule XLIII 268 269 pl 4 figs 122-125 1934 235

Blochwitz in Ann Mycol 33 240 1935 identifies this with *A violaceo fuscus* Gasperini 231

A *atrovirens* Karst in Symb 26 28 Sacc Syll 10 524 Inadequately described for identification

A *aurantiacus* Berkeley in British Fungi fasc IV 1843 Cited by Montagne in Ann Sci Nat Bot 3 Ser 12 299 1849

Syn *Nematogonium aurantiacum*

S *aurea* Greco in Origine des Tumeurs et Mycoses Argentines Buenos Aires pp 671-694 figs 418-428 1916 Not since recognized Some *A ochraceus* strain

A *aureoglaucus* Roburg in Hedwigia 70 137 1930 Culture some strain of *A repens* contributed by Roburg Noted as some member of *A glaucus* group by Blochwitz in Ann Mycol 33 240 1935

A *aureus* Berkeley in English Flora 5 316 1836 The golden yellow elliptical conidia reported by Berkeley suggest *A citrisporus* but actual identification from the description is impossible 219

A *aureus* Nakazawa in Inst Gov t Res Formosa Rept Vol 1 1907 219

A *aureus* varieties described by Nakazawa Simo and Watanabe in Jour Agr Chem Soc Japan No 144 1936 (In Japanese)

var *acidus* 220

var *brevis* 220

var *minor* 220

var *murinus* 220

var *pallidus* 220

comparison of figures and measurements given convince the authors that these varieties could not be safely identified by descriptive data

S auricoma Gueguen in Bull Soc Mycol France 15 1 1 157 figs 1–48 1899 In A *ochraceus* group

A auriculari e Moquin Tandon in Elements Bot Med 2 ed p 466 1866 This name was not accompanied by adequate de scription to separate the form intended from other species which are occasionally found in the ear 276

A aviarius Peck in N Y State Mus Rept (1890) 44 120 1892 A strain of *A fumigatus* isolated from a canary

A arenaceus George Smith in Brit Mycol Soc Trans 25 24 27 Pl 1 figs 1 3 1943 151

A awamori Nakazawa in Inst of Gov t Research Formosa Rept 1 1907 and 2 1912 A member of the *A niger* group 246

A awamori varieties described by Nakazawa Simo and Watanabe in Jour Agr Chem Soc Japan No 141 1936 (In Japanese) 220

var fe rugineus

var fumeus 220

var fuscus 220

var minimus 220

var piceus 220

No doubt the describers had a series of industrially significant strains but it is doubtful if others could identify them from the description and figures given 220

A awamori Usami in Myk Centralbl 4 193 1914 Species recognized by Mosseray in La Cellule 43 264 1934 With *A awamori* Nakazawa cited as a synonym

A bainieri Mosseray in Ann Soc Sc Bruxelles 54 ser B p 79 1934

Syn *A longobasidia* Bainier fide Mosseray in La Cellule XLIII (2) 227 1934 233

A barbae Castellani Cited by Sartory A in Champignons parasites de l homme et des animaux p 505 1922 This organism was found in the beard of a native of Uganda and again from Ceylon The only further information given is conidia 4 to 5μ globose dark brown 233

S basidiosepta Sartory Sartory and Meyer in Ann Mycol 27 317– 320 Pl VII 1929 A variant of the *A candidus* group 211

A batatae Saito in Centralb f Bakt 2 Abt 18(1/2) 34 1907 In the *A niger* group 225

A belfantii Carbone in Atti d Inst Bot Univ Pavia Serie II Vol XIV 63 fig 11 1914 Probably in *A glaucus* group

A (Sporodinia) *bellomontii* Montagne in Ann Sci Nat Bot Ser 4 V 12 181 182 1859 Noted as related to *A maximus* (Sporodinia) Not an Aspergillus

S bicolor J Ray in Rev Sc Ser 4 V 8 176–177 193 212 Lille 189 Probably in *A sydowi* series

A biourgei Mosseray in La Cellule XLIII 211 212 pl 4 figs 81–85 1934 One of the *A niger* group 192

S blanc jaune Bainier nomen nudum A culture so labeled from the Bainier Collection represents a diminutive member of the *A candidus* group 234

A blochwitzii Biourg nomen nudum listed in Biourge MS (p 3) among the *A clavatus* group as a synonym of *A clavatus var gigantea* Blochwitz 211

A boedijni Blochwitz in Ann Mycol 32 83–89 1934 Syn *A terreus var boedijn*

E bonariense Speg in Anal de la Soc Cient Argen 10 174 1880 Some member of the *A glaucus* group but data are inadequate 197

Coemium Bolzianum Saccardo See *A versicolor*

A bouffardi Brumpt (1905) Cited by Castellani and Chalmers in Man Trop Dis p 805 1913
Syn *Madurella Bouffardi* (Brumpt) Dodge Med Myc p 685 1935

A Brodeni (Mattlet) Dodge in Dodge Med Mycol p 635 1935 170
Syn *S Brodeni* Mattlet in Ann Soc Belge Med Trop 4 167–171 figs 1 2 1924 Probably *A unguis*
var *Vancampenhouti* (Mattlet) Dodge in Dodge Med Myc 636 1935

A bronchialis Blumentritt in Ber Deut Bot Ges 19 442–446 pl 22 figs 1–6 1901 also ibid 23 419–427 pl 19 figs 1 3 6 7 8 19 and 23 1905 In the *A fumigatus* group 151

A brunneo fuscus See in Les Malades du papier piqué Paris p 29 1919 Unidentified except as a member of the *A glaucus* group

A brunneo virens Delacroix in Bul Soc Myc France 13 120 with text figure 1897 Unidentified except as a member of the *A glaucus* group

A brunneus Delacroix in Soc Mycol Bul France 9 185 Pl XI fig III 1893 Delacroix described this as the conidial stage of *A echinulatus* q v 131

S Buntingiana Biourge nomen nudum listed in Biourge s MS as applied to a culture of a member of the *A candidus* group received from Bunting

A Buntingii Mosseray in La Cellule XLIII 236–238 pl 4 fig 91–95 1934 Member of *A niger* group in Biourge Collection 1939 233

A butyracea (Bainier) n comb 282
Syn *S butyracea* Bainier in Bull Soc Bot France 27 29 1880 C Roumeguere s Fungi Gallici Exsiccati No 995 is recorded as Bainier s material In the *A ochraceus* group 282

A byssoides Sprengel in Sys Veg ed 16 V 4 541 1827 A fungus on rotting paper with globose fuscous heads but not identifiable

A cacao This name appeared upon a culture in the Bainier collection (Thom No 4640.397) and has been contributed also by Pribram (4777 4) The organism is a strain of *A tamarii* 266

A caesiellus Saito in Jour Coll Sci Imp Univ Tokyo 18 49 Pl III fig 14 1904 Regarded by Neill (in Royal Soc New Zealand Trans 69 242 1939) as *A restrictus* G Smith 141

A caespitosus n sp Raper & Thom in Mycologia 36 563–565 fig 3 1944 A member of *A nidulans* group 166

A calyptratus Oudemans in Arch Neerl II 7 283 pl 13 1902 See *A fumigatus* group 151
var *italicus* Ferraris in Ann Mycol 10 294 1912

S cameleo Sartory Sartory and Meyer in Ann Mycol 29 360–361 Pl III 1930 Some variant of *A sydowi* 186

S candida Sacc in Michelia 1 p 91 1877 In the *A candidus* group

S candidula Bainier in Sacc Syll 4 73 1886
Syn *S candida* Bainier in Bul Soc Bot France 27 30 1880 A member of the *A candidus* group

A candidus Link in Obs p 16 1809 207
var *thermophilus* Nakazawa et al in Jour Agr Chem Soc Japan 88 17 1932 (in Japanese) cited Blochwitz Ann Mycol 33 244 1935

A capitulo pullo Haller in Historia Stirpum Indigenarum Helvetiae

Inchoata etc 1768 Apparently taken from Micheli 1729

S carbonaria Bainier in Bul Soc Bot France 27 27–28 1880 See *A carbonarius* (Bainier) Thom In *A niger* group

A carbonarius (Bainier) Thom in Jour Agr Res 7 12 1916 209

A carbonarius seu ate Meis and Parascandalo in Gaz Ospedali 16 769–772 1895 Cited by Dodge in Med Myc 6 0 as not an Aspergillus 229

A carneolus Sacc in Michelia 1 p 77 1877 And Fungi italici No 18 In *A glaucus* series

A carneus (van Tieghem) Blochwitz in Ann Mycol 31(1/2) 81 1933

Syn *Sterigmatocystis ca nea* van Tieghem in Bull Soc Bot France 24 103 1877 See also Saccardo Sylloge 4 4 and Wehmer s Monograph (Mem Soc Phys His Nat Gen pp 1 157) 1899–1901 Species in *A te eus* group 201

var *subglobosa* Blochwitz in Ann Mycol 33 243 1935 201

var *opaca* Blochwitz in Ann Mycol 33 249 1935

A carnoyi Biourge descr Thom and Raper in U S Dept Agr Misc Pub 426 p 34–5 1941

S castagnei Biourge undescribed culture in the Biourge Collection listed as near *A carbona ius* in the *A niger* group 134

A castanea Patterson in Bul Torrey Bot Club 27 284 1900 In *A tamarii* series

A cellulosae Hopffe in Centralb f Bakt etc 1 abt 83 531–37 1919

Syn *A fumigatus*

A cervinus Massee in Kew Misc Bul 4 158 1914 Neill considers *A gratioti* Sartory to be a synonym but that is not probable 151

A chevalieri (Mangin) Thom and Church in The Aspergilli p 111 1926

Syn *E chevalieri* Mangin in Ann Sci Nat Bot Ser 9 10 361–362 fg 12 1909 In the *A glaucus* group 118

A chevalieri var *intermedius* Thom and Raper in U S Dept Agr Misc Pub 426 p 21 fg 8B 1941 121

A chevalieri var *multiascosporus* Nakazawa Takeda Okada and Simo in Agr Chem Soc Japan Jour 10 135–192 1934 Received from Baarn and appears as NRRL No 88 A strain of *A chevalieri*

F chilense Montagne in Syll Crypt No 919 Fl Chil VII p 4 6 cited by Saccardo Syll 1 p 27 In the *A glaucus* group 190

S chlo ina Cooke and Massee in Grevillea 18 7 1889 Probably a nonascosporic strain of the *A glaucus* group

A ch ysospermum Thaxter nomen nudum attached to a culture later shown to be *A citrisporus* Van Hohnel q v

A chungii Shih in Lingnan Sci Jour 15(3) 378 1933 In the *A flavus* group

A churchii Mosseray in La Cellule XLIII 242 244 pl 4 figs 76–80 1934 Representative of the *A niger* group In the Biourge Collection 1939 96

A cimme ius Berkeley and Curtis in Grevillea 3 108 1875 This specimen was identified by Dr Farlow (Bibliographical Index I pt 1 page 277) as *Periconia chlorocephala* Fres 234

A cinerescens Bainier and Sartory in Bul Soc Mycol France 27 98–101 pl III figs 6–12 1911 In the *A glaucus* group

A cine eus Spegazzini in Anal Soc Cient Argen 10 162–163 1880

A disjunctus Bainier et Sartory in Bul Soc Myc France 27 346–368 pl X–XI 1911 A member of the *A glaucus* group See *A echinulatus* 133
A diversicolor Waksman in Soil Science 2 126 1916 The reference to an organism by Waksman under this name was a typographical error for *A versicolor*
S dubia (B & Br) Sacc in Fungi italici pl 902 and Sylloge 4 72 1886
Syn *A dubius* Corda in Berkeley and Broome in Ann Nat Hist 2 Ser 7 100 (No 590) 1851 Not thus far recognized
A dubiosus Lindau in Rabh Krypt Fl 8 151 1907 In the *A candidus* group
A eburneus Biourge nomen nudum was attached to a culture received from Biourge and recorded as NRRL 515 It is accepted as *A niveus* 202
A echinosporus Sorokin Abst by Busch in Ztschr f Pflanzenkrankheiten 3 155 1893 Ref in Sacc Sylloge 11 592 1895 The description suggests a Haplographium
A echinulatus (Delacr) Thom and Church in The Aspergilli p 107 1926 131
Syn *E echinulatum* Delacr Soc Mycol de France Bul Trimest 9 266 Pl XIV fig III 1893
Syn *E verruculosum* Vuill in Soc Mycol de France Bul Trimest 34 83 1918 In *A glaucus* group 131
A effusus Tiraboschi in Ann di Bot 7 (fasc 1) 16 1908 See also Thom and Church in Am Jour Bot 2 109–110 1921 Described originally from rotten corn (*Zea Mays*) belongs to the *A flavus-oryzae* group 267
A elatior Mosseray in La Cellule XLIII 253 255 pl 3 figs 29–32 1934 In the Biourge Collection 234
A elegans Gasperini in Atti Soc Toscana Sci Nat Pisa Mem 8 (fasc 2) 328 188 In *A ochraceus* group 281
E epixylon Kunze and Schm (D Schw No 83 Wallr Fl Crypt No 2078) In Compendium Florae Germanicae 4 331 1833 This was evidently some member of the *A glaucus* group
A erythrocephalus Berkeley and Curtis in Jour Linn Soc [London] Bot 10 362 1869 In *A tamarii* group 257
Inzengaea erythrospora Borzi in Jahrb Wiss Bot (Pringsheim) 16 450–463, pls 19–20 1884 Manifestly *A variecolor* 163
A exiguus Hann
Syn *A conicus* fide Blochwitz in Ann Mycol 31 73 1933
S ferruginea Cooke in Grevillea VIII (1879) 95 in Jour Quekett Mic Club 2 Ser 2 139 1885 Not an Aspergillus Republished in Veg Wasps and Plant Worms p 184 1892 see also Petch in Trans Brit Myc Soc XVI p 72 1931 who examined type specimens as rust colored patches on pupae conidiophores hyaline smooth 12µ diam vesicle globose 50µ primary sterigmata 30 by 4 to 12µ secondary 14 18 by 7 0µ conidia from 9 by 5µ to 6 to 9µ subglobose brown coarsely rough a brown member of the *A niger* group
A ferrugineus Fuckel in Fungi rhenani No 157 also Symbolae Mycologicae in Jahr d Nassauischen Vereins fur Naturkunde Jahrg XXIII and XXIV 3–8 1869–70 Not identified
A ferrugineus Link in Sp Plant Ed 4 6 pt 1 p 68 1824 Also in Fries Sys Myc 3 p 387 1829 Both authors seem to have had members of the *A glaucus* group
A ficuum (Reich) Hennings 325

Syn *Sterigmatocystis ficuum* (Reich) I Henn in Hedwigia 34 Heft 2 86 1895
Syn *Ustilago ficuum* Reichardt in Verhandl K K Zool Bot Gesell Wien 17 335 1867 A culture distributed under this name (Thom 14?) is a rapid oxalic acid producing organism In the *A niger* group

A fiemonthi Sopp cited as a name on a culture by Biourge in Ms p 19 in the *A flavus* group

A fimetarius Peck in N Y State Mus Bot Rept 4? p 128 1889 Probably *A candidus* group

A fimeti Sacc in Michelia 2 543 188? Inadequately described

A fischeri Wehmer in Centralb f Bakt II abt 18(12/15) 390-39? figs 5 1907 In the *A fumigatus* group 151
Syn *A fumigatus-ascosporic* see Thom and Church in Am Jour Bot 5 91-9? 1918

A flavaureus Biourge nomen nudum listed in his Ms as a member of the *A flavus-oryzae* group with yellow-orange reverse as shown by a culture in his collection

A flavescens Wreden in Compt Rend Acad Sci Paris 65 368 186 Also St Petersb Med Zeitschr 13 133 1867 Identifications have not been satisfactory Possibly in *A nidulans* group

A flavidus Berkeley and Broome in Jour Linnean Soc (London) Bot 14 101 1875 Cited by Saccardo in Fungi of Ceylon London 1871-3 No 913 b Probably a member of the *A flavus* group but not more closely identifiable

A flavipes (Bainier and Sartory) Thom and Church in The Aspergilli p 155 1926 1?9
Syn *S flavipes* Bainier and Sartory in Bul Soc Mycol France 27 90-96 pl III figs 1-6 1911 179

A flavo-viridescens Hanzawa in Journ Coll Agr Tohoku Imp Univ Sapporo 4 23? 3 II 21 figs 1-4 1911 In *A versicolor* group 19?

A flavus Link in Obs p 16 1809 (H F Link Observationes in Ordines plantarum naturales Gesellschaft Naturforschender Freunde zu Berlin Magazin 3 1809—commonly cited Link Obs) 263

A flavus forma *Maydis* Ciferri R in Ann Mycol 20 46 1922

A flavus var *japonica* cited by Blochwitz in Ann Mycol 31 ?4 1933 without description

A flavus var *viridis* Blochwitz in Ann Mycol 32 86 1934 Is vaguely designated as a non tropical strain apparently duplicating *A parasiticus* Speare 267

A flavus mut *rufa* Blochwitz in Ann Mycol 27(3/4) 201 1929 A mutation which shows gradations to pure brown from the green form

A flavus mut *fusca* Blochwitz in Ber d Bot Ges 48 5 6 1928

Eurotium Aspergillus flavus DeBary and Wor Terminology employed by DeBary and Woronin (Beitr z Morph u Physiol d Pilze III Reihe p 380 1870) to designate relationship of the asexual species *A flavus* with species characterized by a perfect stage and included in *Eurotium* 263

A foetidus n sp 219
Syn *A aureus* Nakazawa A black Aspergillus with a strong musty odor 219

A fonsecaeus n sp
Syn *S fusca* Bainier in Bul Soc Mycol France 27 29 pl 1 fig 5 1880 A black Aspergillus on

Session Tunis pp 601-603 1913 Some one of the A *glaucus* group 133

A gracilis Bainier in Bul Soc Myc col France 23 92 Pl IX figs 11-14 1907 In A *restrictus* series 138

var *exiguus* Bainier & Sartory in Bull Soc Mycol France 28 47 pl 2 1912 According to the description this variety differs in physiological characters slightly from A *gracilis* Bainier 140

A granulatus Mosseray in La Cellule XLIII 249-50 pl 3 figs 25-28 1934 A member of the A *niger* group in the Biourge Collection 234

A granulosus Raper and Thom in Mycologia 36 565-568 fig 4 1944 In the A *ustus* group 175

A gratioti Sartory in Compt Rend Acad Sci (Paris) 170 523-524 1920 Also in Champignons parasites de l homme et des animaux pp 578-579 1922 Probably some member of the A *fumigatus* group 154

A Greconis Dodge in Dodge Med Myc p 634 1935 Syn *S aurea* Greco q v

A griseus Link in Sp Plant Ed 4 6(1) 69 1824 Incorrectly cited by Bonorden and Wehmer as Link Obs 1 69 1809 Name also used by Fries and by Bonorden Not identified

A guequeni was figured in Biourge s monograph of the Penicillia (La Cellule t 33 fasc 1 pp 7-330 1923) as *P guequeni* Plate XX He afterward distributed it as A *guequeni* In A *restrictus* series 139

A guttifer Mosseray in La Cellule XLIII 235-236 pl III fig 53-57 1934 In the Biourge Collection 233

A gymnosardae Yukawa in Jour Coll Agr Tokyo I 362 Pl 18 figs 1-7 1911 This fungus was found by Yukawa under the name *awokabi* and is described by him as essential to the ripening of the tunafish preparation katsuobushi The dimensions given are intermediate between those of A *flavus* and A *oryzae* and closely approximate those of A *pseudoflavus* Although we have cultures related to these forms we have not been able to identify these intermediates except as members of the A *flavus-oryzae* group 266

A hageni Hallier in Cattaneo Mico Corp Um p 123 Pl 6 fig 8 Florentin 1892 146

Syn *Otomyces Hageni* Hallier in Zeitschr Parasit 1 195 1869 2 22 233 and 259 Pl 5 1870 Not identifiable

A halophilus Sartory Sartory and Meyer in Ann Mycol XXVIII 362-363 Pl III figs 11-14 1930 Probably some nonascosporic member of the A *glaucus* group 118

A helicophorus nomen nudum attached by Thaxter to a culture that was found to belong with A *ustus* 174

S helva Bainier in Bul Soc Bot France 28 78 1881 In A *ochraceus* group 281

A hennebergi Blochwitz in Ann Mycol 33 238 1935 Regarded by Neill as one of the A *ochraceus* group See A *wentii* group 249

A herbariorum species name seems to appear first as *Mucor herbariorum* in Primitiae Florae Holsaticae by Fredericus Henricus Wiggers 1780 republished in Facsimile edition No 23 by W Junk 1925 See A *glaucus* group 100

Eurotium herbariorum ser *minor* Mangin in Ann des Sci Nat Bot (Ser 9) 10 365 1909 See A *mangini* 127

Eurotium herbariorum ser *major* Mangin in Ann Sci Nat Bot

(Ser 9) 10 365 1909 A glaucus group characterized by large ascospores Would fall within A echinulatus q v 131

A heterocephalus Spring in Bull Acad Sci Belg 19 568 1852 This name was given to colonies in a hen's egg which showed small heads globose and large heads columnar Since no adequate figure or description was offered it may be disregarded as a nomen nudum

Phycomyces heterosporus Harz See A atro ruber

P (Micro-aspergillus) Hickeyi figured by Biourge in La Cellule XXXIII fasc 1 1 331 1923 (Penicillium Monograph) proved to be indistinguishable from A gracilis q v 139

A hispidulus Sprengel in Sys Veg Ed 16 4 541 1827 There is no suggestion of an Aspergillus in the description given

A holmensis Biourge nomen nudum listed among the A versicolor group in Manuscript and in culture in his collection in 1930

A hortai (Langeron) Dodge in Dodge Med Mycol p 628 1935 204

Syn S hortai Langeron in Bul Soc Path Exotique 15 383-384 figs 1 3 1922 204

Syn A terreus Thom 195

A humicola Chaudhuri and Sachar in Ann Mycol 32 97 1934 Cited by Neill as A versicolor 193

A humus Abbott in Ia St Coll J Sci 1 15-36 Fig 9a-e 1926 See also Louisiana Station Bul 194 One of the A ustus series 174

A hypojanthinus Biourge was published as Penicillium hypojanthinum in Biourge Monogr La Cellule 33 fasc 1 pl 391 ? Pl XXII fig 130 1923 Afterward he transferred it to Aspergillus A restrictus series 139

A incrassatus Spring in Bull Acad Roy Belg 19 ... fig ? 18.. Not identifiable

E insigne Winter (ex siccati in Rabh Fungi No 1737) in Hedwigia 1873 and Rabh Krypt Fl Aufl 1 Abt 2 2 61 188. Probably not an Aspergillus

S insueta Bainier in Bull Soc Myc France 24 85-87 Tab VIII figs 1 13 1908 A ustus series 174

A intermedius Speg in My Arg V in An Mus Nac Buenos Aires Ser 3 T 13 435 1911 In the A niger group

A itaconicus Kinoshita in Bot Mag Tokyo 45 60-61 1931 Species diagnosis and figure reprinted in Acta Phytochim (Tokyo) 5(3) ? 1 ? 1931 See A glaucus group 14

S italica Sacc in F italici N 100 1881 Also in Michelia 1 ... 1877

Syn A sterigmatophorus Sacc in Atti d Soc Ven Tren d Sc Nat 2 fasc 2 232 Tab I fig 5-8 1873 Some member of the A candidus group 11

A janus n sp Raper and Thom in Mycologia 36 556-561 fig 1 1944 187

var brevis Raper & Thom in Mycologia 36 561 563 fig ? 1944 190

A japonicus Saito in Bot Mag Tokyo 20 61-63 1906 In A niger group 231

var capillatus Nakazawa Takeda and Suematu Culture available in Centraalbureau 1939

var or mut grisea Blochwitz in Ann Mycol 33 240 1935 It was a change of name only A maliaceus Mosseray

A javanicus cited by Takahashi and Sakaguchi in Jour Agr Chem Soc Japan 1 No 10 1925 only in parenthesis after A fumaricus Wehmer and apparently deemed a synonym In A niger group

A jeanselmei Ota in Ann de Parasitologie 1(2) 137–146 1923 *A flavus* group 269

A keratitis Ball in Amer Med 2 31 1901 This organism was found in an ulcer in the human cornea Not identifiable 147

A koningi Oudemans in Arch Neerl Ser II V 7 284 Tab XIV 1902 Not since identified

A laneus Link Obs p 16 1809
Syn *Botrytis lanea* (Bonorden) Sacc in Syll 4 74

A laneus in Schweinitz in Syn Am Bor is syn for *Rhinotrichum curtissii* Berk in Grevillea 3 108 1875

A laokiashanensis Shih in Lingnan Sci Jour 15(3) 368 1933 Near *A unguis* in the *A nidulans* group 169

E laterītium Montagne in Century VI No 35 Ann Sci Nat Bot 3 Ser XI 154 1849 Sylloge p 257 One of the *A glaucus* group with ascospores 7 to 10μ in diameter

A Lepidophyton Wehmer
Syn *Epidermophyton concentricum* fide Dodge Med Myc 490 1935

A lignieresi Cost and Lucet in Ann Sci Nat IX Bot 2 137 Pl 5 figs 19–23 1905 This culture from the lung of a penguin differs in cultural details from typical *A fumigatus* especially by the presence of swollen groups of cells in the mycelium 159

A longobasidia Bainier nomen nudum a culture so labeled (Thom 4640 477) was received from the Bainier Collection through da Fonseca This was the type of *A bainieri* Mosseray in Ann Soc Sc Bruxelles 54 ser B p 72 1934 Member of the *A niger* group characterized by very long primary sterigmata 253

A loiantiensis Biourge nomen nudum culture distributed by Biourge and appearing in NRRI Collection as No 76 See Thom and Raper U S D A Misc Publ 426 p 18 1941 117

A luchuensis Inui in Jour Col Sci Imp Univ Tokyo 15 469 Pl 22 figs 1 8 1901 230
var *rubeolus* Shih in Lingnan Sci Jour 15(3) 374 1933 230

S lutea Bainier in Bul Soc Bot France 27 27 1880 *A flavus* group See also Sartory and Jourde in Compt Rend Acad Sci (Paris) 146 548 549 1908 269

A luteus (van Tieghem) Dodge in Dodge Med Myc p 625 1935
Syn *S lutea* van Tieghem in Bul Soc Bot France 24 103 1877

A luteo niger (Lütz) Thom and Church in The Aspergilli p 166 1926 226
Syn *S luteo nigra* Lutz in Bul Soc Bot France 53 48 52 1907 Collected by A Chevalier at San Thome Africa in fermenting seeds of *Theobroma cacao* In the *A niger* group 226

A luteo niger van Luijk nomen nudum in Biourge s MS p 15 Probably attached to a culture of some black Aspergillus

A luteo virescens Blochwitz in Ann Mycol 31(1/2) 73 83 1933 In *A tamarii* series 256

A lutescens Bainier nomen nudum described by Thom and Church in The Aspergilli p 193 1926 In *A tamarii* series 251

A lutricolor Biourge nomen nudum listed in MS among the *A ochraceus* group in the Biourge Collection

A Macfiei Dodge in Dodge Med Mycol p 269 1935
Syn *Sterigmatocystis sp* Macfie in Ann Trop Med Parasitol 15 2 9 281 1921 Pathogenic ? In the *A niger* group 239

A macrosporus Bonorden in Handbuch d allg Myk 1851 fig 193 Not identifiable

A malignus Lindt in Arch Exp Path Pharm 25 256-271 figs 1 11 1889 In *A fumigatus* group

A malvaceus Mosseray in La Cellule XLIII 265-266 Pl 4 figs 131-135 1934 A member of the *A niger* group In the Biourge Collection 153

A mangini 235

Syn *A mino* (Mangin) Thom and Raper in U S D A Misc Publ 426 p 27 1941

Syn *E herbariorum* ser *minor* Mangin in Ann Sci Nat Bot (Ser 9) 10 365 1909

Member of the *A glaucus* group with ascospores of intermediate size

A maximus Link in Obs p 16 1809 Not an Aspergillus 127

A maydis Quevedo in De Agronomica Nos 8 and 9 Buenos Aires 1912 See also Sartory in Champignons parasites de l homme et des animaux p 532 1922 A species probably belonging to the *A glaucus* group described in connection with disease in horses

Emericella medias Chowdhury and Mathur in Ann Mycol 36 61-63 1938 Probably *A variecolor* q v 147

A medius Meissner in Bot Ztg 55 336-344 354-357 1897 See Thom and Raper U S D A Misc Publ 426 p 33 1941 163

A melleus Yukawa in Jour Coll Agr Tokyo 1(3) 366 Taf XVII 1911 In *A ochraceus* group 133

A menciei Sartory et Flament in Compt Rend Soc Biol Paris 83 114 115 1920 See also Sartory et Bailly in Les Mycoses Pulmonaires et leur Parasites 279 (Paris) pp 180-181 1923 *A glaucus* group not since reported 146

A michelii Preuss in Linnaea 25 6 1852 Not recognized

A microcephalus Mosseray in La Cellule XLIII p 225-227 Pl 3 fig 42-48 1934 Member of the *A niger* group In the Biourge Collection 233

A microsporus Boke The description and figures given by Cattaneo and Oliva in Arch Lab Bot Critt Garovaglio 5 123 Pl 6 fig 9 1888 have been seen Not recognizable

A micro viridi-citrinus Costantin & Lucet in Ann Sci Nat Bot IX(2) 158 1905 In *A flavus* group

A minimus Wehmer in Bot Centralb 80 449-461 1899 See also Wehmer s Monogr p 79 1899-1901 Wehmer s culture was lost It has not been identified since 263

S minor Bainier in Bul Soc Bot France 27 30 1880 Not recognizable Possibly *A sydowi?*

A mino (Mangin) Thom and Raper in U S Dept Agr Misc Pub 426 p 27-29 1941 See *A mangini*

A minutus Abbott in Louisiana Sta Bul 194 1926 Also in Iowa St Coll Jour Sci 1 15-36 figs a b c and d 1926 See *A ustus* group 127

A miyakoensis Nakazawa Simo and Watanabe in Agr Chem Soc Japan Jour 12(9) 963-964 1936 See *A niger* group 1 4

A mollis Bainier and Sartory in Bul Soc Mycol France XXVII 453 Pl XVI 1911 Not *A mollis* Berkeley See *A glaucus* group 220

A mollis Berkeley in English Flora V pt 2 p 340 1836 Not identifiable 129

A mongolicus Biourge nomen nudum Biourge distributed under this name a culture essentially like *A echinulatus* (q v) It appears in the NRRL Collection as No 137 See Thom and Raper U S D A Misc Publ 426 p 33 1941 132

A monteuidensis Talice and MacKinnon in Soc Biol [Paris] Compt Rend 108 1007-1009 1931 See Thom and Raper U S D A Misc Publ 426 p 26 1941 In *A glaucus* group 125

A mouthoni Biourge nomen nudum listed by Biourge among his Les Furotium in unpublished Manuscript Probably in culture in his collection

A mucoroides Corda in Icones fungorum II 18 fig 76 1837 Probably some member of the *A glaucus* group—unidentifiable

A mucoroideus Cook in Grevillea XII 9 1883 See *A cookei* Sacc

A mulleri Berkeley in Jour Linn Soc XIII 175 1873 Not identifiable

A mutabilis Bainier et Sartory in Bul Soc Mycol France 27 458 pl XVII 1911 In *A glaucus* group 129

A mycetomi Villabruzzi and Gelonesi in Annali Med Nav Colon 33 283 308 8 figs 192?

Syn *Madurella sp* undeterminable from the data available

A mycobanche Link in Sp Plant Ed 4 6 65 1824 A fungus from rotting Peziza—not identifiable

A nantae Pinoy in Compt Rend Soc Biol 97(19) 67-68 192? This was probably *A unguis* in the *A nidulans* group Listed by Biourge as in his collection 1939 1?0

A nanus Montagne in Sylloge Generum Specierumque Crypt p 300 No 1112 Paris 1856 Also in Saccardo Sylloge Fungorum 4 p 71 Patavii 1886 In the *A niger* group 231

A nanus Oudemans, in Nederl Kruidk Arch Ser 3 2 1191 1904 Not *A nanus* Mont q v Probably some conidial form in the *A glaucus* group

E nebulosum Fries in Sys Myc 3 334 1832 No data are given to link this with Aspergillus

A nicollei Pinoy cited by Biourge in 1939 manuscript He has apparently raised to species rank *S nidulans* var *nicollei* Pinoy q v 170

A nidulans (Eidam) Wint in Rab Krypt Fl 12 62 1884 156

Syn *S nidulans* Eidam in Cohn Beitr Biol Pfeanzen 3 39?-411 Pl 20-22 1883

forme *Cesarii* Pinoy in Bul Soc Path Exot 8 11 1915 1?0

mut *coerulea* Blochwitz nomen nudum upon a culture in the Biourge Collection 1939 Listed in Biourge MS

mut *alba* F Yuill in Jour Bot (London) 1939 p 1?5 pl 618 159

var *latus* Thom and Raper in Mycologia 31(6) 65 9 1939 159

var *Nicollei* Pinoy in Compt Rend Acad Sci Paris 144 396 1907 This variety was found fruiting within human tissue in a subject affected with Madura foot Listed in Biourge s Collection 1939 as *S nicollei* Pinoy 1?0

Syn *S nidulans* var *Nicollei* Pinoy in Archives d Parasitologie 10 437-458 Pl XI 1906

Diplostephanus nidulans (Eidam) Langeron in Compt Rend Soc Biol Paris 87 343 315 19??

Syn for A nidulans Eidam q v

A niger van Tieghem in Ann Sci Nat Bot Ser 5 V 8(4) 240 1867 See also Thom and Currie in Jour Agr Res 7 1 15 1916 156

Syn *S nigra* van Tieghem in Bul Soc Bot France 24 102 103 1877 216

Syn *Aspergillopsis nigra* (v Tieghem) Speg in Myc Arg V in Ann Mus Nac Buenos Aires (ser 3) 13 435 1911

mut *cinnamomeus* n comb See *A cinnamomeus* Schiemann

var *laevis* Blochwitz in Ann Mycol 33 249 1935 Proposed for smooth spored strains 223

niger var *fermentarius* Nakazawa Simo and Watanabe in Jour Agr Chem Soc Japan 144 171 2 and 184 1936 (in Japanese)

mut *fusca* Blochwitz in Ann Mycol 32 8 1934 In the *A niger* group Such a separation based upon conidial color is of doubtful validity 220

mut *Schiemanni* n comb See *A schiemanni* (Schiemann) Thom 224

forma *Tuebingen* Schober in the Centraalbureau is apparently the strain used in the researches of Schober

A niger citricus Wehmer name on a culture received from Neuberg (C T No 4668 4) but without description

Syn *A citricus* Mosseray q v

S nigra Bainier Syn for *S phoenicis* (Corda) Patouillard and Delacroix in the *A niger* series 235

A nigrescens Robin in Histoire Naturelle des Vegetaux Parasites (Paris) p 518 pl 5 fig 2 1853 No success has been made in interpreting Robin s organism 151

A nigricans Wreden in Compt Rend Acad Sci (Paris) 65 368 1867 Probably *A niger* group

A nigriceps B and C in the Curtis Collection Specimen collected by Charles Wright (No 927) in Cuba Cited by Cooke M C in Grevillea 17 21 Sept 1888 A slide from this material prepared by Bullard and preserved in the Harvard collection shows a characteristic organism of the *A niger* series not separable from *A niger* van Tieghem

A niveocandidus Lindau in Rabh Krypt Fl 8 151 1900 In the *A candidus* group

A niveo glaucus Thom and Raper in U S Dept Agr Misc Pub 426 p 35–36 1941

A niveus Blochwitz in Ann Mycol 27(3/4) 205–206 Taf III fig 2 1929 A member of the *A terreus* group 135

var *major* Blochwitz in Ann Mycol 32(1/2) 86 1934 Apparently belongs in the *A candidus* group 205

var *nubila* Blochwitz in Ann Mycol 32(1/2) 85 1934 See *A carneus* 211

A Nolting Hallier in Zeitschr Parasit (Not found) cited by Cattaneo and Oliva in Arch Lab Bot Critt Garovaglio 5 122 1888 Not recognizable 202

A notus nomen nudum attributed to Wehmer culture in the Centraalbureau at Baarn was identified by Thom and Raper as *A pseudo glaucus*

E obliteratum Schw Syn fungorum in Amer Bor N 2745 Some unidentifiable member of *A glaucus* group 111

A oblongispo us E and E No 760 in Nuttall s Flora of Fayette County West Virginia Listed apparently by Millspaugh in The Living Flora of West Virginia in W Va Geol Survey

5(A) 32 1913 as A *glaucus* var *oblongisporus* F & W Examination of this material shows it to be a mixture of A *flavus* and A *repens*
S *ochracea* Brunier in Bul Soc Bot France 28 78 1881 Some member of the A *ochraceus* group
S *ochracea* Delacroix in Bul Soc Mycol France 7 109 Pl VII fig f 1891 282
Syn *S delacroixii* Sacc in Sylloge 10 5?7 Delacroix described his organism without recognizing the previous use of the specific name 282
S *ochracea* (Wilhelm) Schroter See A *ochraceus* Wilhelm
A *ochraceo ruber* Sacc in Michelia I 77 1877 (Saccardo P A No 1063 in Myco Veneta on bark of Walnut 1876 see fig 17 in Fungi italici) Some conidial form of the A *glaucus* group
A *ochraceus* Wilhelm Inaug Diss Strassburg p 66 1877 279
var *microspora* Tiraboschi in Annali di Botanica 7 14 1908 281
S *ochroleuca* Speg in Anal Mus Nac Buenos Aires Ser 3 t 13 431 1911 In the A *ochraceus* group 276
A *ochroleucus* Haller in Enum Method Stirp Helvet Indig t I p 6 1742 Hist Stirp t III 1768 Cited by Casperini as questionable syn for A *elegans*
A *okazakii* Okazaki in Centralb f Bakt 2 abt 42(10 14) 225 1914 A *candidus* group 211
This is cited in the Sylloge 22 1260 as *S okazakii* (Saito) Saccardo
A *oligosporus* Corda in Icones III Tab II Not an Aspergillus
S *olivacea* van Tieghem in Bull Soc Bot France 2 103 1877 Not identified with A *olivaceus*
Preuss 1852 Not identifiable
A *olivacco fuscus* Mosseray in La Cellule XLIII 2?8 ?59 pl 3 fig 49-5? 1934 A member of the A *niger* group in the Biourge Collection 231
Cladosarum olivaceum Yuill and Yuill in Trans Brit Myc Soc 22 194 ?00 Pl 11-13 1938 73
A *olivaceus* Delacroix in Bul Soc Mycol France 13 118 1?0 text fig 189?
Syn A *delacroixii* Sacc and Sydow q v Not A *olivaceus* Preuss 185? Probably in A *glaucus* group
A *olivaceus* Preuss in Linnaea 25 77 1852 Not identifiable
A *Oniki* on a culture from Okunuki in the Centraalbureau collection No description found Blochwitz cites it in Ann Mycol 33 240 1935 As A *ochraceus*
A *oösporus* Wallroth as Flora Cryptogamica Germaniae No 19?8 in Compendium Florae Germanicae 4 2?6 1833 Some one of the A *glaucus* group
A *oriolus* nomen nudum attributed to Biourge Distributed by Biourge Collection and appears as NRRL No 87 It was identified as a strain of A *chevalieri* by Thom and Raper in U S D A Misc Publ 4?? pl 21 1941 1?0
A *oryzae* (Ahlburg) Cohn in Jahresb Schles Ges für Vaterl Cultur 21 2?6 1883 In the A *flavus-oryzae* group ?01
Syn *E oryzae* (Ahlb) Korschelt Name with incomplete description of sake organism published in Dinglers Polytech Jour 230 330 18?? ?61
A *oryzae* var *basidiferens* Constantin and Lucet in Ann Sci Nat Bot IX (?) 1? 1905 In the A *flavus-oryzae* group
Syn A *oryzae* var *basidifer* Sacc Syll 2?

A. ostianus Wehmer in Bot Centralb 80 449-461 1899 Monogr pp 11 119 Taf II No I 1899-1901 In the *A. ochraceus* group 283

A. ovalispermus Link in Obs II p 37 1816 also in Sp Plant Ed IV Vol VI(1) 66 1824 Cited as synonym of *A. roseus* Wallr (1833) without evidence of identity or reason for redescription

A. panamensis Raper and Thom in Mycologia 36 568 572 fig 5 1944 242

A. parasiticus Speare in Hawaiian Sugar Planters Exp Sta Path & Physiol Ser Bul 12 p 38 Pl 3 and 4 1912 In the *A. flavus* group 266

Syn *A. flavus* var *viridis* Blochwitz q v 267

A. penicillatus Greville in Scottish Cryptogamic Flora 1 39 plate 3 1823

Syn *A. glaucus* (conidial) not identifiable to species

A. penicillatus Link in Sp Plant Ed 4 6(1) 69 1824 The description given by Link is not sufficient to identify any form but since he probably intended to cover the organism of Greville it may be assumed to be conidial *A. glaucus* Sturm cites it as *Briarea elegans* without specifying his reasons

A. penicilloides Spegazzini in Rev Agrar Veter La Plata p 245 1896 In the *A. restrictus* series 14

A. penicillopsis (Hennings) Racib P Hennings as *Stilbothamnium penicillopsis* P Henn & E Nym described in Fungi Monsunensis (Warburg Monsunia Bd I p 37 (Leipzig) 1899 Exsiccati of type in Pathological collections U S Dept Agr Bur of Plant Industry as Raciborski No 87 in Crypt Paras Java Sceal o Paras Alg u Pelz Javas II 7 1900 282

A. periconioides Sacc in Ann Mycol 11 320 1913 No 195 in Sydow *Fungi exotici exsiccati* collected by P W Graff on leaves of Carica in Luzon 1912 Not identifiable

A. perniciosus Inui in Jour Coll Sci Imp Univ Tokyo 15 473 1901 Inui recorded a yellowish greenish color in the mycelium of this species which is regarded as related to *A. luchuensis* and *A. niger* 230

A. pertalis is a nomen nudum on a culture distributed by Biourge The organism was close to *A. restrictus* series 142

A. petiolatus Haller in Historia stirpum indigenarum Helvetiae inchoata etc 1 68

A. phaeocephalus Durieu and Montagne in Fl Alg p 31 1849

Syn *S. phaeocephala* (Dur et Mont) Saccardo Fungi italici fig 903 and No 1244 in Sacc Myc Veneta 1877 One of the *A. niger* group

A. phoenicis (Corda) Thom in The Aspergilli p 175 1926 222

Syn *S. phoenicis* (Corda) Patouill and Delacr in Bul Soc Mycol France 7 119 Pl 9 1891 223

Syn *Ustilago phoenicis* Corda in Icones Fungorum 4 9 pl 3 fig 26 1840 One of the *A. niger* group 223

A. pictor Blanchard cited by Castellani & Chalmers in Man Trop Med p 806 1913

Syn *Trichophyton picto* Blanchard in Traite Path Gen II 919 1896 The production of polychromatic cultures suggests relationship to *A. versicolor*

A. pollinis Howard in Am Bee Jour 36 577 578 1896 See also idem 38 530-531 1898 This was *A. flavus* fide Turesson

in Svensk Bot Tidskr 11 30 1917 266

S polychroma Ferraris in Fl It Crypt Hyph p 640 1906 193
Syn *A versicolor* q v 190

A polychromus De Mello in Jour Indian Bot 1(5) 158-161 1920 The data given are not sufficient to distinguish whether he had *A nidulans* or *A sydowi*

A polychromus Sartory Sartory and Meyer Cit Syn of *A versicolo* fide Blochwitz in Ann Mycol 31 73 1933

A polymorphus Moquin Tandon in Elements Bot Med 2 Ed 469 1866 Not identifiable

A pouchetii Montagne in Ann Sci Nat Bot 4 Ser 6(12) 182-183 1859 As described this was one of the mucors noted as having resemblances to *A maximus* (Sporodinia)

A praecox Mosseray in La Cellule XLIII 229 1934 Cited as synonym of *A fuliginosus* Peck 233

S prasina Bainier in Bull Soc Bot France 27 31 1880 Not identifiable

A profusus Hann nomen nudum Cited by Thom and Raper in discussing *A scheelei* Bainier and Sartory (Bul Soc Myc France 28 257 1912) as nearly related to *A scheelei* See Thom and Raper U S D A Misc Publ 426 p 13 1941 Syn of *A pseudoglaucus* 111

A proliferans Geo Smith in Brit Mycol Soc Trans 26(1/2) 26 Pl III 1943 In the *A ruber* section of the *A glaucus* group 117

A pseudo carbonarius (Bainier) Mosseray in La Cellule XLIII 224 225 Pl 3 fig 7-13 1934 233
Syn *S pseudo carbonaria* nomen nudum on culture (Thom 4640 482) from the Bainier collection

A pseudo citricus Mosseray in La Cellule XLIII 278-279 pl 4 figs 103-104 1934 Member of the *A niger* group in the Biourge Collection 233

A pseudo-clavatus Puriewicz in Schrift Naturforch Gesell Kiev 16 2 p 309 1900 See Saccardo Sylloge Fung 16 p 1028 In *A clavatus* group 98

A pseudo-elatior Mosseray in La Cellule XLIII 255-256 pl 3 figs 33-37 1937 Member of the *A niger* group in the Biourge Collection 1939 234

A pseudoflavus Saito in Centralb f Bakt 2 abt 18(1/3) 34 figs 15-18 1907 266
Syn *S pseudoflava* Sacc Sylloge Fungorum 22 1260-1266 The morphology given indicates that *A pseudoflavus* is one of the intermediate forms which bridge the gap between typical *A flavus* and *A oryzae*

A pseudoglaucus Blochwitz in Ann Mycol 27 207 1929 Emended description by Thom and Raper in U S D A Misc Publ 426 p 12 1941 *A glaucus* group 110

S pseudo nidulans Vuillemin Arch Parasitologie 8 540-542 1904 Vuillemin transfers the ascosporic form described by Grijns as *A fumigatus* in Centralbl Bakt II 11 330 1903 to this specific name emending Grijns's description by indicating the double nature of the band by which he separates his form from *A nidulans* as described by Eidam This discussion by Vuillemin tallies with the commonest of our American soil forms of *A nidulans* but not with the description by Grijns

A pseudo niger Mosseray in La Cellule XLIII 256-258 Pl 4 figs 113-117 1934 A member of the *A niger* group in the Biourge Collection 1939 234

S pseudo nigra Costantin and Lucet in Bul Soc Mycol France 19 33-44 1903 In the *A niger* group

A pseudo Schiemanni Biourge nomen nudum cited from Centraalbureau catalogue 1931 as an actively diastatic organism represented in the Biourge Collection

A pulchellus (Speg) Thom and Church in The Aspergilli p 181 1926 229

Syn *Aspergillopsis pulchella* Speg in Myc Arg V An Mus Nac Buenos Aires Ser 3 T 13 436 1911 In the *A niger* group 229

E pulcherrimum Winter in Rabh Krypt Fl 2 aufl 1 Abt 2 60 1887 Noted there as also in Herbarium of Winter and in Hansen Fungi fimicoli Danici 1041 of Sep Abdr A coprophilus form from the dung of foxes in Leipzig and dogs in Denmark by Hansen not an Aspergillus

A pulmonum hominis Welcker Discussed by von Dusch in Virchow s Archiv (N F 1) 11 561 566 1857 Apparently *A fumigatus* 151

A pulverulentus (McAlpine) Thom in Jour Agr Res 7 10-11 1916 223

Syn *S pulverulen a* McAlpine in Agr Gaz N S Wales 7 302 1896 In the *A niger* group 223

A pulvinatus B and C original collection as far as seen appears to be in the Curtis Collection from Society Hill S C 1855 also one marked F cub —Wright No 642 in the Curtis Collection another series of specimens of B and C No 1648 in Ellis and Everhart N A *Fungi collected* on dead twigs at Newfield N J 1885 Also No 2306 in the Ellis Collection Not an Aspergillus

S purpurea van Tieghem in Bul Soc Bot France 24 101 103 1877 Possibly *A nidulans*

A purpureofuscus Fries in Sys Myc 3 388 1829 Probably the same as *A purpureofuscus* of Schweinitz

A purpureofuscus Schweinitz in Synopsis fungorum in America boreali media degentium Secundum observationes In Trans Amer Phil Soc N S 4 287 No 2680 1834 Also in Saccardo Sylloge Fungorum 4 68 Patavii 1886 Not an Aspergillus

A purpureus Haller Historia stirpum indigenarum Helvetiae inchoata etc 1 68 Not *recognizable*

S pusilla Peyronel in I genu atmospherici dei funghi con micelio Thesis Padova p 21 1914 See the *A niveus* series 203

A pusillus Massee in Kew Bull Misc Inf 4 158 1914 From Soil Sudan Not identifiable

A pyri English nomen nudum name published in Research Studies of the State College of of Washington VIII (3) 127 1940 (Doctoral Thesis Taxonomic and pathogenicity studies *of the fungi which cause decay* of pears in Washington) Subsequent work by English led to recognition of the name as a synonym of *A niger* 231

A quadrifilus Link in Obs 2 36 1816 Probably not an Aspergillus

A quadrilineatus Thom and Raper in Mycologia 31(6) 660 fig 3D and 4B 1939 160

A quercinus (Bainier) Thom and Church in The Aspergilli p 186-187 1926 276

Syn *S quercina* Bainier in Bul Soc Bot France 28 78 1881 276

A quininae Heim in Bul Soc Myc France 9 239 1894 The culture was found upon quinine solution but the description given will not separate it from *A fumigatus*

A racemosus Persoon in Neues Mag Bot 1 121 1794 Also in Tentamen Disp Meth Fung p 41 1797 Not recognizable

A ramosus Hallier in Ztschr f Parasit 2 266–269 Pl 6 figs 1 6 1870 The figures and descriptions evidently represent a strain of *A fumigatus* 151

A raulini nomen nudum in Biourge s table probably attached to a culture in his collection

A rehmii Zukal in Oesterr Bot Zeitschr 43 160 Pl II figs 1–10 1893 Zukal regarded this form as close to *S sulphurea* Fresenius but his description of perithecia and ascospores excludes the *A ochraceus* group Blochwitz evidently believed that Zukal had a mixed culture hence the name would be untenable Cultures belonging to the *A ochraceus* group have been distributed under the name but without proving their authority 251

A repandus Bainier and Sartory in Bul Soc Myc France 27 463 Pl XVIII 1911 *A glaucus* group 133

A repens (Cda) DeBary in Abhandl d Senkenberg Naturf Gesellsch 7 359 1870 103

A repens DeBary and Woronin in Beitrage zur Morphologie und Physiologie der Pilzen p 379 1866

Syn *A glaucus* var *repens* Corda in Icones 5 53 Taf II fig 21 1842 103

E repens var *amstelodami* Vuill Soc Mycol de France Bul Trimest 36 131 1920 172

A restrictus G Smith in Jour Text Inst 22 T 115 fig 5 1931 141

A restrictus var B C Smith in Jour Text Inst 22 T 115 figs 4 6 and 8 1931 See *A restrictus* series in *A glaucus* group 141

A roseus Batsch in Elenchus Fungorum p 183 No 58 fig 58 1783 Cited by Link Spec Pl ed IV t VI pt 1 p 68 1824 Also by various authors for a rosy or flesh colored organism and by Corda as *Haplotrichum roseum* in Pracht flora Pl VI

A roseus Link in Sp Plant ed IV t 6 part 1 p 68 1824 Link took the name roseus used descriptively but not nomenclatorially by Batsch (El Fung p 183 no 58 fig 58 1783) for a mold presumed to have been an Aspergillus by later authors The name appears as No 2794 in the Curtis Collection (1849) for a member of the *A candidus* group Neither descriptions nor specimen dating back to these authors fix this name for any definite series

A rubens Green in Boston Soc of Med Sc 1868 Not identifiable

A ruber (Bremer) 114

Syn *A ruber* (Spieckermann and Bremer) Thom and Church in The Aspergilli 112 1926 114

Syn *Eurotium rubrum* Bremer in Zeitschr f Untersuch d Nahrung und Genussmittel IV 1901 p 72 also in Die fettverzehr Organismen in Nahr u Futtermitteln Dissert Munster 1902 114

Syn *E rubrum* Spieckermann and Bremer in Landw Jahrb 31 81–128 1902 114

A ruber Estienne

Syn *Physomyces heterosporus* Harz

Syn *S rubra* (Estienne) Biourge
Syn *Monascus purpureus* certainly not an Aspergillus

S rubescens nomen nudum on a culture in the Bainier Collection (Thom No 4640 48) *A flavipes* group 1

A rufescens Berlese in Fungi Moricolae Fasc VII No 4 Tav 54 figs 12 16 1889 Probably conidial strain of *A glaucus* group 191

A rugulosus Thom and Raper in Mycologia 31(6) 661 2 fig 3F and 4C 1939

A rutilans Mosseray in La Cellule XLIII 234 235 Pl 4 fig 80–90 1934 A member of the *A niger* group in the Biourge Collection 1939 160

A sacchari Spegazzini in Anales del Museo Nacional de Buenos Aires 6 Ser 2 3 244 1899 Some insufficiently described member of the *A glaucus* group 233

A sacchari Chaudhuri and Sachar in Ann Mycol 32 95 1934 Blochwitz in Ann Mycol 33 240 1935 leaves this species in *A quercinus*

A salmoneus Biourge nomen nudum cited by Henrard in La Cellule XLIII fasc 2 p 3.3 1934 as one of the series glauci On p 3 0 idem he records that single ascospores required 3 months for germination and that this species was found to be homothallic Identification to species within the *A glaucus* group is not possible without more information 278

Alliospora Sapucaya Pim in Proc R I Acad 1883 and in Jour Bot 1883 p 234 One of the *A niger* group causing rot in alliaceous bulbs

A sa to yi nomen nudum was attached by Biourge to a culture of an organism close to *A gracilis* in the *A restrictus* series 9

A sartoryi Sydow in Ann Mycol 11 156–160 II VIII 1913 Probably in *A tamarii* group 139

A scheelei Bainier and Sartory in Bul Soc Myc France 28 2?– ?? II X 1912 See Thom and Raper in U S D A Misc Publ No 426 p 12 1941 *A glaucus* group 4

A scheelei Bainier and Sartory var *B ...le...* See *A repens* series in *A glaucus* group 10

A schiemanni (Schiemann) Thom in Jour Agr Res 7 13 1916 See also *A fuscus* Schiemann name changed because previously used

S schneggiana Biourge nomen nudum in Biourge's MS listed in the *A candidus* group as applying to a culture received from Schnegg 2

A sclerotiae Mosseray in La Cellule XLIII (fasc 2) 24 248 1934 A member of the *A niger* group 233

A sclerotiorum Huber in Phytopathology 23(3) 306–8 fig 1 1933 A heavy sclerotium producing member of the *A ochraceus* group

A sejunctus Bainier et Sartory in Bul Soc Myc France 27 346–368 Figs XXI 1911 An ascosporic form of the *A glaucus* group 278

E semiimmersum Marchal in C R Soc R y Bot Belg 33 128 Pl II fig 3 1893 Not an Aspergillus 114

A siebenmanni Costantin and Lucet in Ann Sci Nat Bot 9 2 p 162 1905 This name is based upon Siebenmann's description of an organism from the human ear identified by Siebenmann as *A flavus* but regarded by the describers as a separate species

based upon the description given by Siebenmann 266

A *simplex* Persoon in Tent Disp Meth Fung p 41 1797 Tradition calls it a Penicillium

A *soya* on a culture from Okunuki in the Centraalbureau collection No description found cited by Blochwitz in Ann Mycol 33 240 1935 as A *flavus*

S *skottsbergii* Bresadola and Vestergren no 250 in Vestergren Micromycetes rariores selecti Rossia baltica ins Osilia Kiel kond in silva abiegna prope Kattiel in foliis vivis Aquilegiae vulgaris 1899 Distributed without description examined in collaboration with Professor Thaxter at the Harvard University Cryptogamic Herbarium did not prove to be an Aspergillus

A *spadix* Amons in Arch v Suiker industrie in Nederlandsch Indie 29 12 14 1921 This is one of the A *tamarii* series 257

A *sparsus* Raper and Thom in Mycologia 36 572-574 fig 6 1944 283

A *sphaerospermus* Corda in Icones II 18 1854 No description

A *spiralis* Grove in Journal of Bot 23 164 tab 257 fig 5 1885 A conidial organism of the A *glaucus* group

A *spirius* cited by Amons in Arch v d Suikerindustrie in Nederlandsch Indie 29 14 1921 Not identifiable

S *spuria* Schroeter in Cohn Kryptogamen Flora von Schlesien 3 2 Hälfte Lief 1 p 218 1893 201

Syn ? S *carnea* van Tieghem 1877 See A *carneus* (van Tieghem) Blochwitz 201

A *stellatus* Curzi in Rend Acad Naz Lincei 19 424-428 fig 1 1934 Culture in Centraalbureau list 1939

Syn A *variecolor* 163

F *stercoraria* Hansen in Meddelelser fra den Naturhist Foren i Kjobenhavn p 310 1876

Syn *Anixiopsis stercoraria* Hansen in Bot Ztg 55 127 131 Tab II fig 8 1897

A *stercoreus* Sacc in Michelia 1 75 1877 Also Fungi italici no 19 An unidentifiable member of the A *glaucus* group

A *sterigmatophorus* Saccardo in Atti Soc Ven Tren Sci Nat 2 fasc 2 232 Tab XVII fig 5-8 1873 211

Syn S *italica* q v 211

A *strychni* Lindau in Hedwigia Bd 43 Heft 5 306-307 1904 223

Syn A *pulverulentus* McAlpine 1896 In the A *niger* group 223

A *subfuscus* Johan Olsen in Meddelelser fra Naturh forening i Kristiania 1885 Cited also in O Johan Olsen Videnskapselkabet i Kristiania p 21 1886 Some member of the A *niger* group 231

A *subgriseus* Peck in Bul Torrey Bot Club 22 5 210 1895

Syn F *subgriseum* Peck in Rept N Y State Mus Bot p 30 1910 Also in N Y State Mus Bul 150 1911 There is no way to identify Peck s species

A *sulphureus* (Fres) Thom and Church in The Aspergilli p 185-186 1926 275

Syn S *sulphurea* Fresenius in Beitr / Mykologie Heft 3 p 83 Tab XI fig 30-33 1863 Also Sylloge 4 73 1886 In A *ochraceus* group 275

A *sydowi* (Bainier and Sartory) Thom and Church in The Aspergilli p 147 148 1926 181

Syn S *sydowi* Bainier et Sartory in Ann Mycol 11 25-29 11 III 1003 A *sydowi* series 181

var *achlamydosporus* Nakazawa Sim and Watanabe in Jour Agr Chem Soc Japan 10(3) 1 8-1 9 1934 The absence of

Hülle cells is inadequate for separation

A syncephalis Cueguen in Champ Parasit Hom Anim p 165 fig 6 1904 Probably *A fumigatus* 186

S szurakiana Moesz in Bot Közlem 19 44-66 13 figs 1921 One of the *A candidus* group 151

A tabacinus Nakazawa Simo and Watanabe in Jour Agr Chem Soc Japan 10(2) 177 178 1934 Appears to have been a strain of the *A versicolor* series

A tamarii Kita in Centralb f Bakt etc 2 Abt 3 No 14/16 pp 432-452 1913 *A tamarii* series 193

A tardior Bourge nomen nudum listed among his cultures of the *A restrictus* group (Bourge s Sec I Microaspergilli) 251

A terreus Thom in Turesson Cote Svensk Botanisk Tidskrift 10 5 1916 Without description diagnosis Thom and Church Amer Jour Bot 5 85-86 1918 The *A terreus* series

var *aureus* n var 195

var *Boedijni* (Bloch) n comb Thom and Raper 198
Syn *A Boedijni* Blochwitz Ann Mycol 32(1/2) 83 1934

var *floccosus* Shih in Lingnan Science Jour 15 372 pl 16 fig 3 1936 197

var *subfloccosus* Shih in Lingnan Science Jour 15 371 pl 16 fig 4 1936 198

A terricola Marchal in Rev Mycol 15(59) 101-103 1893 In *A tamarii* series

var *Americana* Marchal in Thom and Church Am Jour Bot 8 125 1921 Also in Thom and Church The Aspergilli p 192 1926 253

A thomii This was an undescribed sclerotium producing strain of *A flavus* sent by P W Graff to Amer Type Cult Collection 253

A tiraboschii Carbone in Atti d Inst Bot Univ Pavia Ser II Vol XIV 320 1912 In the *A sydowi versicolor* group 266

A tokelau Wehmer in Centralbl Bakt I 35 140 1903 Cited by Dubreuihl in Jour Med Bordeaux 32 312 1902 Wehmer s culture was one of the *A glaucus* group but not the pathogenic organism causing the disease tokelau Dodge Med Myc 490 1935 calls it Epidermophyton 186

S tropicalis Matta in Bol Inst Brasil Sci 3 51 54 2 figs 192? Probably *A sydowi* but not separable from other strains

A tubingensis (Schober) Mosseray in La Cellule XLIII 215-21? pl 3 fig 58-60 1934 Member of the *A niger* group in the Bourge Collection 1932

A tunetanus (Langeron) Dodge in Dodge Med Mycol p 635 1935
Syn *S tunetana* Langeron in Bul Soc Path Exot XVII 315-34 illust 1924 See *A sydowi* series

A umbrinus Patterson in Bull Torrey Bot Club 25 284 1900 In the *A tamarii* series 18

A umbrosus Bainier and Sartory in Bul Soc Mycol France 28 267 Pl XII 1912 In the *A glaucus* group

A unguis (Emile Weil and Gaudin) Emend Thom and Raper in Mycologia 31(6) 667 8 fig 6 1939 179
Syn *S unguis* Emile Weil and Gaudin in Arch Med Exp Anat Path (Paris) 28 463-465 1919 See Thom and Raper The *A nidulans* group Mycologia 31 667 1939 169

A ustus (Bainier) Thom and Church in The Aspergilli p 152 153 1926 169

Syn *S usta* Bainier in Bul Soc Bot France 28 78 1881 *A ustus* group 171
var *laevis* Blochwitz in Ann Mycol 32(1/2) 84 1934 175
A ustilago Beck in Itin Prin S Coburgi 2 148 (Wien) 1888 Saccardo in Sylloge Fungorum 10 526 (Patavii) 1892 223
Syn *A phoenicis* in the *A niger* group 222
A Vancampenhouti Mattlet in Ann Soc Belge Med Trop 4 167–176 1924 Name only *ibid* 6 31 1926 In the *A versicolor* group 193
S varia Bainier in Bul Soc Bot France 27 30 1880 Probably some nonascosporic *A nidulans* or *A unguis*
A variabilis Gasperini in Atti Soc Toscana Nat Sci Pisa Mem 8 fasc 2 p 326 1887 From the description this was probably some strain of the *A flavus* group 266
A varians Ceni in Rivista sperimentale di Freniatria p 31 1905 Certainly identified by Tiraboschi in Annali di Bot 7 9–10 1908 as *A versicolor*
A varians Wehmer in Bot Centralb 80 460–1 1899 Also in Wehmer Monogr pp 77–79 taf I 1899–1901 If Wehmer's description is correct his species is not known in culture now The authors have believed that *A varians* is identical with *A itaconicus* 142
A variecolor (Berk and Br) Thom and Raper in Mycologia 31 663 667 fig 4D and fig 5 1939 163
Syn *Emericella variecolor* Berk and Br in Introd Crypt Bot p 340–341 fig 76 185 See Patouillard in Bull Soc Myc Fr 7 43–49 pl 4 fig 6–12 1891 163
Syn *Inzengaea erythrospora* Borzi Jahrb Wiss Bot (Pringsheim) 16 450–463 pl 19 20 (1884) 1885 163
Syn *Emericella medias* Chowdhury & Mathur Ann Myc 36 61–63 1938 163
Syn *A stellatus* Curzi Rend Acad Naz Lincei 19 p 424–428 fig 1 1934 163
A variegatus Mosseray in La Cellule XLIII 238–239 pl 4 fig 72–75 1934 Member of the *A niger* group, in the Biourge Collection 1939 233
A velutinus Mosseray in La Cellule XLIII 252 253 pl 3 fig 38–41 1934 Member of the *A niger* group in the Biourge Collection 1939 234
S veneta Massalongo in Bul Soc Bot Ital No 7–8 p 159 1900 Not cultivated and not identifiable by description although Werkenthin (Phytopathology 6 247–249 1916) used *A venetus* for strains now known to be *A terreus*
E verruculosum Vuillemin in Bul Soc Myc France 34 83 1918 See *A echinulatus* in *A glaucus* group 131
A versicolor (Vuillemin) Tiraboschi in Ann Bot (Rome) 7 9 1908 190
Syn *S versicolor* Vuillemin cited by Mirsky in Thèse de Med Nancy No 27 p 16 1903 *A versicolor* series 190
mut *coerulea* Blochwitz in Ann Mycol 27(3/4) 201 1929 Represents a pure blue strain arising from a green strain Probably *A sydowi*
var *fulvus* Nakazawa Takeda and Suematu culture available from the Centraalbureau 1939
var *glauca* Blochwitz in Ann Mycol 32 86 1934 A strain greener than typical for the species from human skin accompanying a Trichophyton Similar green strains have been observed by us 192

A violaceo cited by Biourge in his MS p 16 but no data offered

A violaceo fuscens (Was this *A violaceo fuscus*?) mut *grisea* Blochwitz in Ann Mycol 32 87 1934 The grounds of separation are vague

A violaceo fuscus Casperini in Atti Soc Toscana Sci Nat Pisa Mem 8 fasc 2 p 3 6 1887 In *A niger* group

A virens Link in Obs Ord Pl Nat p 16 1809 also Sp Pl Ed 4 6 pt 1 p 67 1824 This has never been surely identified 231

A virens (Link?) Fischelbaum in Verh Naturw Ver Hamburg 3 Folge XII p 31 1906 This might have been close to *A ficheri*

Syn *E virens* (Fischelb) Sacc Saccardo cites description of preceding under this name in Sylloge Fung 22 1255 1913

A viridans nomen nudum attached by Biourge to a culture distributed by him One of the *A restrictus* series

A viridis apparently undescribed appears only on a specimen in the Curtis Cc llection now in the Cryptogamic Herbarium of Harvard University the label is *A viridis* Schw in herb *A virens* Lk in Syn N S Herb Schw No Aspergillus could be found in the specimen 140

A viridi griseus Costantin and Lucet in Ann Sci Nat Ser IX 2 140 1905 One of the *A fumigatus* group

S vitellina Ridley in Jour Bot 34 157 Plate 35 figs 14 16 1896 No one has since reported Ridley's organism with certainty but a culture belonging in the *A ochraceus* group appears under the name in C B S list Blochwitz regarded it as a synonym for *A flavus* 151

A vulpinus nomen nudum was distributed by Biourge It belonged to *A tamarii* Kita 276

S wehmeri (Bresadola) Hennings Cited by Wehmer in Centralbl f Bakt etc 2 Abt 18 394 395 1907 Examination of material from Hennings by Wehmer furnished no basis for separating the form from *A niger* Examination of similar specimen in the Farlow Herbarium shows primary sterigmata up to 60 by 12μ which would place it near *A phoenicis* 206

Syn *Ustilago wehmeriana* Bresadola

A wehmeri Costantin and Lucet in Ann Sci Nat Bot Ser IX 2 16 1905 The name is proposed by Costantin and Lucet for the organism which Brefeld and Wehmer described as *A flavus* Link and which is so used in this paper The uncertainties in the identification of Link's species do not seem important enough to justify the change of name *A wehmeri* is to be regarded as a synonym of *A flavus* q v

A wentii Wehmer in Centralbl Bakt 2 Abt 2 150 1896 Schimmelpilze als Wehmer Die Pilzgattung Aspergillus etc in Mem Soc Phys d Hist Nat Geneva 33 part 2 p 119 1899 1901 266

var *minimus* Nakazawa Takeda Okada and Simo culture available from the Centraalbureau in 1939 246

A westendorpii Saccardo and Marchal in Rev Mycol 7 149 1885 In *A clavatus* group 49

Chapter XXV

ACCEPTED SPECIES, VARIETIES, AND MUTATIONS

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A. amstelodami (Mang.) Thom and Raper 122
A. atropurpureus Zimmerman 226
A. avenaceus G. Smith 246
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A. butyracea Bainier 282
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A. candidus Link 207
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A. clavatus Desm. 9[illegible]
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